1979

Aluminum and Manganese in the Etiology of Grass Tetany.

Vivien Gore Allen
Louisiana State University and Agricultural & Mechanical College

ALLEN, VIVIEN GORE

ALUMINUM AND MANGANESE IN THE ETIOLOGY OF GRASS TETANY

The Louisiana State University and
Agricultural and Mechanical Col. PH.D. 1979

ALUMINUM AND MANGANESE IN THE ETIOLOGY OF GRASS TETANY

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Agronomy

by
Vivien Gore Allen
B.S., University of Tennessee, Martin, 1962
M.S., Louisiana State University, 1973
December, 1979

ACKNOWLEDGEMENTS

The author wishes to acknowledge the many individuals who have contributed their ideas, assistance, and encouragement to this research. Appreciation is extended to the Department of Agronomy, Dr. W. H. Willis, Head, Emeritus, and Dr. J. Preston Jones, Head, for this opportunity for study and research. Gratitude is also extended to the members of my committee of which Dr. Donald L. Robinson, Associate Professor of Agronomy, is chairman. Special acknowledgement is extended to Dr. Robinson for his guidance and encouragement during the preparation of this manuscript.

The nature of this research is such that an interdisciplinary approach is critical to its development. Consequently, I am particularly indebted to Dr. F. Glen Hembry, Professor of Animal Science, who served as minor professor and who has at all times gone out of his way to render me the utmost cooperation, assistance and encouragement.

Also deserving of particular acknowledgement is Dr. B. J. Miller, Associate Professor of Agronomy. Although Dr. Miller is not a member of this committee, the study of aluminum and its possible relationship to grass tetany was included in this research at his suggestion. Indications of a conformation of this association were not found in the literature until this project was close to completion. Appreciation is also extended to Dr. Miller for his encouragement, his assistance, and the use of his laboratory for analyses.

Finally, I would also like to express my indebtedness to the following:

To Dr. Joseph E. Sedberry, Jr., Professor of Agronomy, for providing his laboratory and equipment for analyses,

To Mr. S. A. Lytle, Associate Professor of Agronomy, Retired, for his technical assistance in the identification of the soils in the grass tetany and research pastures,

To Mr. R. H. Brupbacher, Professor and Mr. Rodney Henderson, Associate, Department of Agronomy, Soil Testing Laboratory, for their assistance in conducting the chemical analyses,

To Dr. Edward P. Dunigan, Professor of Agronomy, for providing his laboratory and advice in conducting the microbiological research,

To Dr. H. M. Selim, Associate Professor of Agronomy, for technical assistance in conducting the controlled soil moisture research,

To Dr. Antonio Achacoso, Associate Professor of Dairy Science, for his encouragement and assistance in designing and conducting the in vitro experiments,

To Dr. William Fairchild, DVM, for his assistance in locating sites for field research,

To the other veterinarians in private practice who assisted in locating and diagnosing grass tetany: Dr. Maxwell Lea, Dr. Donald W. Winkler, Dr. C. Grey McAdams, Dr. David R. Cress, Dr. Jimmy B. Smith, Dr. Larry Knight, Dr. S. F. Fontenot,

To Dr. Steven Nicholson, Specialist, Veterinary Science and Associate Professor of Toxicology, for assistance in contacting veterinarians,

To Dr. Prentiss Schilling, Head, Department of Experimental Statistics, and Mrs. Penny Culley, Research Associate, for the

statistical analysis and assistance in interpretation of the data,

To Mr. and Mrs. Leslie Bickham, Mr. John Barton, Mr. C. B. Griffin, Mr. Sonny Guttziet, Mr. Willie Hurdle, Mr. Raymond Long, and Mr. T. Chalmers McKowen for providing pastures for field plot research,

To the many other cattlemen whose cooperation was essential to this project,

To Mr. Miguel Coelho and Mr. Randy Wright for their assistance with the animal research,

To the Animal Science Department for providing a controlled temperature room for the growth chamber research,

To Dr. Barbara Mosse, Soil Microbiology Department, Rothamsted Experiment Station, for providing the Endogone Sporocarps,

And to my husband and three sons whose patience, understanding and encouragement made possible this period of study and research.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	ix
ABSTRACT	x
INTRODUCTION	1
LITERATURE REVIEW	3
MATERIALS AND METHODS	15
RESULTS AND DISCUSSION	35
DISCUSSION	77
SUMMARY AND CONCLUSIONS	82
APPENDIX	86
LITERATURE CITED	93
VITA	102

LIST OF TABLES

Table		Page
1.	Locations, soil types, soil test values and dates of establishment and harvest of field plots	18
2.	Analysis of profile samples of Falaya silt loam and Mhoon silty clay loam soils used in growth chamber and greenhouse experiments.	21
3.	Analysis of the Falaya silt loam and Mhoon silty clay loam surface (0-10 cm) soils used in the growth chamber and greenhouse experiments.	22
4.	Soil water content of Falaya silt loam and Mhoon silty clay loam determined by the pressure plate method at four suction values	22
5.	Analysis of profile samples taken at sites A, B, C, and D in the Bickham Research Pasture	31
6.	Mineral analyses of forage samples from grass tetany pastures in Louisiana, 1977-79.	36
7.	The correlation coefficients between soil moisture levels and mineral concentrations in forages from grass tetany pastures, 1978 and 1979.	38
8.	Analysis of soil samples taken from grass tetany pastures at the site of highest forage aluminum accumulation.	40
9.	The forage species, soil series at the site of highest Al accumulation and geographic location of grass tetany pastures	41
10.	Mineral analysis of rumen content samples from grass tetany and non-tetany animals in Louisiana, 1977-79 . . .	43
11.	The effects of P and two Mg sources on the mineral concentrations in ryegrass forage of three locations in Louisiana, 1977.	44
12.	The effects of mycorrhizal fungi, soil moisture, and harvest date on mineral concentrations in ryegrass in a greenhouse.	46

Table Page

13. The effects of five levels of soil moisture on mineral concentrations in ryegrass grown in the greenhouse on Falaya silt loam and Mhoon silty clay loam. 48

14. Mineral analysis of ryegrass grown on Falaya silt loam and Mhoon silty clay loam and soil analysis of soil types in the greenhouse experiment with five moisture conditions. 49

15. Observations of the presence of endomycorrhizal fungi in ryegrass roots under five levels of soil moisture in Falaya silt loam and Mhoon silty clay loam in the greenhouse. 52

16. Soil water content in two soil types under five suction values at two depths in greenhouse pots 54

17. The effect of three temperatures on mineral concentrations of ryegrass in a growth chamber 54

18. Mineral concentrations in ryegrass grown in a growth chamber at 8-12 C as a function of time following a rise in temperature to above 14 C 58

19. Effect of two soil moisture levels on mineral accumulations in ryegrass under growth chamber conditions . . 59

20. Correlation coefficients between forage Al accumulations and the recorded variables at four locations in the Bickham research field. 63

21. Correlation coefficients between forage Mn accumulations and the recorded variables at four locations in the Bickham research field. 66

22. The best variable model found in the stepwise regression of Al with temperature and soil moisture variables in the Bickham pasture. 67

23. The best variable model found in the stepwise regression of Al with all variables of temperature, soil moisture, and forage mineral concentrations measured in the Bickham pasture. 68

24. The influence of Al and Mn on the solubility of Mg and Ca in combinations of ryegrass, rumen fluid and buffer solution during in-vitro incubation. 70

25. The chemical analysis of forage samples collected in pastures grazed by animals with a diagnosis of grass tetany. 87

Table Page

26. The chemical analysis of soil samples collected in pastures grazed by animals with a diagnosis of grass tetany. 89

27. Analysis of serum samples from lactating cows using two dilution techniques 91

LIST OF FIGURES

Figure		Page
1.	Daily fluctuations in soil water suction values obtained for 100, 300, and 600 cm treatments in Falaya silt loam.	23
2.	Daily fluctuations in soil water suction values obtained for 100, 300, and 600 cm treatments in Mhoon silty clay loam	24
3.	Soil moisture levels monitored with tensiometers connected to mercury monometers in the greenhouse experiment.	25
4.	The effect of soil moisture level on the concentration of Al and Mg in ryegrass grown in the greenhouse.	51
5.	The effects of cold temperature for 22 days on the accumulation of Al and Mg following a rise in temperature above 14 C in ryegrass grown in a growth chamber	55
6.	The concentration of Al and Mg in ryegrass as a function of time following a rise in temperature to above 14 C in the growth chamber	57
7.	Forage Al concentration, soil temperature and visible moisture status at site D during five month sampling period in the Bickham pasture	60
8.	The effect of adding Al and Mn to the rumen on average serum Mg levels in fistulated steers.	72
9.	The effect of added Al on the concentration of soluble Al in the rumen fluid of fistulated steers.	73
10.	The average effect of orally administered aluminum and manganese on serum Mg levels of lactating cows.	75

ABSTRACT

Grass tetany, a major metabolic disease of ruminants, is characterized as a Mg deficiency. Although several factors are known to contribute to a hypomagnesemic condition, the true cause of this disease remains obscure.

A field investigation was conducted over a three-year period (1977-79) to characterize mineral concentrations in soils and forages under the conditions of grass tetany. Eleven sites, ten in Louisiana and one in Tennessee, were included in the study. Mineral levels in the rumen contents of animals that died from grass tetany were also investigated. Chemical analysis showed that forages were generally low in Mg ($< 0.20\%$), below 3% in K, and high in Al concentration. Aluminum values commonly ranged from 2,000 to 8,000 ppm. One location was found to exceed 14,500 ppm Al. Aluminum levels were highly variable and a wide range of values were found among sampling sites within each pasture. Forages with high Al concentrations grew on many different soil types which ranged in pH from 5.1 to 7.3. Exchangeable Al was not detected in the soil surface horizons (0-8 cm). Conditions of high soil moisture were significantly correlated with high Al accumulation by forages. Rumen contents of dead tetany animals contained an average of 2373 ppm Al, more than five times the level found in normal fistulated cattle grazing under non-tetany conditions.

Grass tetany often occurs on wet soils and following a period of cold temperature, occurring five days after the mean temperature rises above 14 C. A greenhouse experiment with two soils and five

moisture levels showed that ryegrass, Lolium multiflorum, Lam. cultivar Gulf, grown on ponded soil moisture conditions contained higher concentrations of Al, regardless of soil type, than forage grown under drier soil conditions. Growth chamber studies showed that plants exposed to 0-3 C temperatures accumulated more Al following a rise in temperature above 14 C than plants exposed to 7-11 or 8-12 C. Aluminum concentration began a linear increase two days after the rise in temperature and continued through the eight-day sampling period while Mg ceased to increase after day four.

Forage Al concentrations, soil moisture, soil temperature and air temperature were monitored during the tetany season at four sites within a tetany-prone pasture. Aluminum concentrations exceeded 1,000 ppm at only one site, the only site under ponded moisture conditions. This high concentration occurred seven days after the soil temperature rose from 3 C to above 14 C.

It is generally accepted that adult ruminants are highly dependent upon dietary intake and absorption for maintenance of Mg balance. Therefore, an in vitro study was designed to determine the effects of Al and Mn on the solubility of Mg and Ca in various combinations of rumen fluid, ryegrass and buffer solution. Seven treatments were used: control, 1,000 and 2,000 ppm Mn, 4,000 and 8,000 ppm Al, 1,000 ppm Mn plus 4,000 ppm Al, and 2,000 ppm Mn plus 8,000 ppm Al. Levels were calculated as ppm in forage dry matter. After 48 hours of incubation, added Al had reduced Mg in solution by 56% and Ca by 74%. Manganese reduced Ca solubility when added alone but had no effect on Mg or Ca when added with Al. An in vivo experiment was designed to investigate the effect of ingested Al on serum Mg and Ca. Four fistulated steers,

maintained on bermuda grass hay, were used in the Latin Square experiment. Four treatments were administered once daily: control, 4,000 ppm Al, 2,000 ppm Mn, and 4,000 ppm Al plus 2,000 ppm Mn, calculated as ppm of daily feed intake. Serum Mg levels in the Al treated steers dropped within 24 hours after treatments began and declined 32% by the end of four days. After treatments were discontinued, serum Mg levels returned to normal. Manganese had no significant effect on serum Mg level.

INTRODUCTION

Grass tetany is a major metabolic disease of ruminant animals on a cool-season forage diet and is characterized as a Mg deficiency of sufficient magnitude that the animal exhibits clinical symptoms. Although a hypomagnesemic condition can be precipitated by an absolute lack of Mg intake, animals suffering acute symptoms of grass tetany often have a dietary level of Mg that would normally be considered entirely adequate. Supplementing the animal with additional Mg provides a measure of protection but a level adequate for prevention is often difficult or impractical to insure. Subclinical hypomagnesemia may go completely unnoticed. With its potential for limiting the productive capacity of the animal, this may be as important economically as the problem of grass tetany itself.

A great deal is known about the pathology of grass tetany. Researchers have shown a number of factors which affect both the level of Mg in forages and its utilization by the animal. However, no factors or combinations of factors have ever been shown to cause grass tetany to occur in a repeatable and predictable manner. It is suggested by some to have no single causitive agent, being instead the result of an imbalance among several different and variable combinations of nutritional and physiological influences. The possibility remains, however, for the existence of some key, central factor which by itself is sufficient to cause grass tetany. Under field conditions its effect could undoubtedly be enhanced by the presence of one or more contributory factors.

Successful treatment of this disease must lie somewhere between increasing the total amount of Mg available for animal consumption and decreasing the collective counterbalancing influences capable of reducing Mg utilization. Based on this premise the objectives of this research were two-fold: (1) to study the feasibility of raising the Mg status of cool-season forages through the use of phosphate fertilizers and increased plant root infection with endomycorrhizal fungi, and (2) to investigate the concentration of minerals present in forages, soils and rumen contents under the conditions which cause grass tetany. Hopefully these investigations would allow identification of imbalances capable of contributing to depressed utilization of dietary Mg by the ruminant animal. Manganese and Al were studied in a series of greenhouse, growth chamber, field, in vitro and animal experiments in order to elucidate the conditions under which they were accumulated by forages and their potential for interferring with Mg balance within the animal.

LITERATURE REVIEW

The metabolic disorder of ruminant animals known as grass tetany is, as the animal translates it, a magnesium deficiency and is characterized by abnormally low levels of blood serum Mg. Far from a simple lack of Mg intake, the cause of this disorder has been the subject of investigation for over one hundred years.

Grass tetany has been reported in many parts of the world including Great Britain, Germany, Scotland, The Netherlands, Australia, New Zealand, France, Scandinavia, Ireland, Belgium, Argentina, and the United States and is responsible for a considerable portion of the losses from metabolic diseases. Commonly called grass tetany, it is also known as hypomagnesemic tetany, grass staggers, wheat pasture poisoning, lactation tetany, Hereford disease, spring tetany and Kopziekte (11,26,33,69).

Clinical symptoms of grass tetany are those of nervous apprehension, loss of appetite, stiff and stilted movement, staggering when walking, twitching of the muscles, especially about the face and ears, profuse salivation and grinding of the teeth. The third eyelid will protrude or flicker as in tetanus. Violent convulsions may develop within hours or days leading to coma and death. Chances of recovery are much greater if treatment is begun before the animal is in the comatose state. Diagnosis by clinical symptoms alone is difficult. In the field the early symptoms closely resemble those of ketosis, prussic acid poisoning, milk fever and nitrate poisoning. A more positive diagnosis can be made on the basis of serum Mg levels (16,25,78,108,114).

Low blood Mg is a necessary prerequisite condition but animals may be hypomagnesemic without developing clinical symptoms of grass tetany (1,8,33,101). Low serum Mg levels were first related to the disease by Sjollema in the Netherlands in 1928 (94,95). Crookshank and Sims (13) in a study of sixty animals with wheat pasture poisoning found an average serum Mg level of 13.5 $\pm$ 7.5 ppm. Meyer (70) classified animals normal at 18-32 ppm Mg, slightly hypomagnesemic at 12-18 ppm and severely hypomagnesemic at less than 12 ppm. Storry and Rook (99) found serum Mg levels of 10-15 ppm in animals approaching tetany. Pauli and Allsop (81) found the onset of clinical tetany symptoms to be more closely related to low Mg levels in the cerebrospinal fluid than with low serum Mg.

Hypomagnesemia is often accompanied by hypocalcemia, in spite of an apparently adequate intake of both Ca and Vitamin D. Forbes (27) found serum Ca levels in tetany cows significantly lower ($P < 0.01$) than in normal cows, with hypocalcemia present in 88% of the cows diagnosed with grass tetany. Correction of the condition is solely dependent upon restoring Mg. When Mg levels are restored, the hypocalcemia, after a lag period, returns to normal. Infusion with Ca alone results in increased serum Ca only as long as the infusion lasts. It has been suggested that clinical tetany occurs as a result of disturbance of Ca metabolism brought about by severe hypomagnesemia (2,11,19,36,37,41,53,54,60,68,102).

Studies of Mg deciciencies with various species suggest that the level and stage of deficiency influences the etiology of grass tetany. There is an apparent failure to mobilize Ca from the bones, due perhaps to the development of resistance to the parathyroid hormone

(PTH) which is not usually elevated inspite of low Ca blood levels. At certain stages of Mg deficiency in some individuals PTH elevation does occur. However, serum Ca levels remain depressed, indicating a resistance to this hormone. Other subjects have normal or low PTH levels, indicating inadequate production of PTH. There is therefore, a failure of the PTH mechanism at two levels in maintaining Ca homeostasis. It appears that Mg facilitates the release of Ca from bone in the presence of adequate amounts of vitamin D and PTH. After Mg is administered, PTH secretion increases after a lag period which may extend over several days. A rise in serum Ca follows the increase in PTH. This lag presumably is the time required for Mg ions to enter the hydration shell of bone, permitting Ca exchange to begin (15,22,25,67,92).

Under normal conditions a relatively constant level of Mg is maintained in the animal body, primarily by urinary excretion of excesses and avid conservation by the kidneys during times of insufficiency. Magnesium excretion through the kidneys stops when blood levels reach about 18 ppm Mg. Above this renal threshold there is a linear relationship between blood plasma Mg levels and urinary Mg levels. Clinical symptoms of grass tetany have been observed to be preceded by a decrease to zero of urinary Mg (33,35,49,86,99).

The defense against deficiency in ruminants is much less effective than the defense against excess. In the adult, Mg is not mobilized from body reserves (bones) at a sufficient rate to compensate for dietary deficiency, as indicated by sharp drops in both urinary and serum Mg levels when intake is depressed. In young animals, bone Mg can be mobilized to a considerable extent, 30% or more, but the adult

is highly dependent upon intake and absorption (11,68,77). The absorption rate in ruminants is in the order of 5-30% (26,71). Recent work has indicated that the major site of Mg absorption in the ruminant is before the pylorus (2,4,32,50,71,82,85). The work of Tomas and Potter (106) and Field and Munro (24) indicate that most of the Mg absorption occurs in the reticulo-rumen and that the abomasum does not appear to be an important site.

In monogastrics, the principal absorption sites appear to be certain sections of the small intestine. Because of the differences in absorption sites, information on Mg absorption worked out on laboratory animals can not be applied to ruminants (26,71). Martens, et al. (61) suggested that Mg transfer through rumen mucosa can not be adequately explained by simple diffusion and that uptake and transfer may occur by two different and seemingly independent processes.

Grass tetany is most often observed in older, female ruminants in the early stages of lactation. Tetany can occur in lactating animals of any age, however, as well as animals in late stages of pregnancy. Pregnancy increases the animals' Mg requirement about twenty percent and lactation by as much as 2-4 times the daily requirement of the non-pregnant, non-lactating female (43) and is undoubtedly a factor in the etiology of the disease. Ewes with twin lambs are more tetany prone than those with single births (38,109). It has been reported in all European breeds of beef and dairy animals and has been reported in steers (39).

Beeson (3) and Salmon (91) have reviewed soil Mg. Cooper, et

al. (12) have reviewed relationships of soil and plant Mg. Grunes, et al. (33) have reviewed the literature concerning Mg in soils, plants and animals.

Tetany pastures are often characterized as being high in forage levels of K, high in N, below 0.27% Mg, having a K/(Ca + Mg) ratio of greater than 2.2, low in Na, high in trans-aconitate and citric acid, and low in water soluable carbohydrates. [See reviews by Butler (8), Dishington (19), Grunes, et al. (33), Kemp (47), Kemp and 't Hart (48), Mayland, et al. (62), Metson, et al. (69), Stuart, et al. (100) and Voisin (112)].

In general, attempts to relate soil Mg levels to plant Mg levels have been of only limited success. The Mg supplying capacity of a soil may be limited not only by the absolute amount present, but also by insufficient rates of solution, enhanced leaching, or insufficient rate of absorption by plants due to excesses of cations such as K, Na, Mn, Ca, or NH_4 (12,33,56,64). Magnesium fertilizers are generally unsuccessful in raising forage Mg levels as a means of preventing grass tetany. Forages, grown on soils that are deficient in Mg, may respond to Mg fertilizer applications, however (33,63,65).

The work of several investigators indicates that Mg acts as a carrier for phosphorus and that increasing supplies of one will result in an increased plant content of the other (31,90,107). Gillingham and Page (31) found Mg uptake to be increased by NO_3, SO_4 and PO_4 but not by Cl ions. Russell (90) indicated that the P content of a crop can sometimes be increased by adding a Mg fertilizer.

It has been known for some time that the infection of tree roots

by ectotropic mycorrhiza fungi promotes P absorption from soils (7, 34). It has more recently been shown that endotropic mycorrhiza are widespread on crop roots and that their effect on promoting P absorption can be quite large (30,76,105). Foliage content of other elements including N, K, Ca, Na, Mg, Fe, Mn, Cu, B, Zn, and Al has been shown to differ between mycorrhizal and non-mycorrhizal plants (76,88). Mosse (76) reported differences to be inconsistent with the exception of Cu which was generally higher and Mn which was generally lower in mycorrhizal plants. Holevas (42) reported an increase in P and Mg and a lowering of K in the leaves of strawberry plants when the roots were infected with vesicular-arbuscular mycorrhiza.

Vesicular-arbuscular mycorrhiza are present in most habitats and are found in most species of the family Gramineae. The exceptions are grasses that grow in very wet soils. Such plants may become mycorrhizal if they are transplanted into a well-drained soil. Mycorrhizal fungi, as well as other fungi, do not tolerate water-logged conditions. Small grains sown in the fall may not become extensively infected with mycorriza until the following spring (30).

Mosse and her colleagues have shown that differences exist among various strains of mycorrhiza in their ability to promote P absorption and suggest that selection of strains might be a powerful technique for promoting P absorption where P is low in availability (74,75).

Tetany fields are often described as being excessively wet. Temperature also appears to be a factor. Kemp and 't Hart (48) indicated in a study done in the Netherlands, that five days after the mean daily temperature rises above 14C, there is an increase in the number of tetany

cases. 't Hart (103) observed that 95% of the cases he studied occurred when the mean temperature was 8-14 C and indicated that 5-15 C were the limits reported in other parts of the world.

Elkins and Hoveland (20) and Elkins et al. (21) found that the Ca, Mg and K content of ryegrass (Lolium multiflorum, Lam.) increased as soil O_2 increased and that the concentrations were higher in plants grown at 16 C then in those grown at 12 C. The work of Karlen et al. (46) is in general agreement, showing a decrease in Ca and Mg in plant tissue with increasing soil moisture. Potassium increased or remained unchanged. They also found significant differences to exist in Mg uptake among the different cultivars of wheat tested. Leggett et al. (51) found no differences, however,in the concentrations of K, Ca and Mg or in the ratio of K/(Ca + Mg) in tall fescue when the root media was maintained at 12 C over the control group maintained at 25 C.

Since grass tetany in cattle is often associated with early spring forage, typically high in K and low in Mg, Lentz et al. (52) suggested that altering serum Mg and K levels may affect intermediary carbohydrate metabolism resulting in the hypoglycemia and ketosis that are often observed in tetany cases. In further work they suggested that a relationship exists between absorption of Mg and glucose intake (57,72).

Blakemore et al. (6) reported high levels of Mn in grass (540-1320 ppm in dry matter) associated with grass tetany in Lincolnshire. Their experiments with rabbits showed that oral sub-lethal doses of Mn were followed immediately by a reduction of blood serum Mg levels. They produced the same effect in cattle and sheep with Mn administered

by stomach tube. Mean serum Mn levels of six cows were found to rise from 0.01 to 1.5 g/ml, 20 days after the cows were turned out on pasture. A significant decrease in serum Mg also occurred. Fain et al. (23) reported a similar depression in serum Mg in cows receiving a normal ration supplemented with 100 ppm Mn as manganese sulfate, but not at supplement levels of 150 or 200 ppm. Underwood (108) stated that all animal species, except possibly rabbits, are very tolerant to soluble bivalent manganese salts; that it is poorly absorbed and largely excreted in the feces.

Robinson, et al. (84) reported that the ability of rumen microorganisms to digest cellulose in vitro was markedly reduced in animals on high dietary levels of manganese, but found no differences in serum Ca or P values of yearling calves fed rations containing up to 1000 ppm Mn as manganese sulfate. Serum Mg levels were not reported. Cunningham et al. (14) in a similar study also found no effect on serum Mg levels when feeding 4920 ppm Mn. In an in vitro study they observed depressed volatile fatty acid production and marked changes in rumen flora at high levels of Mn intake.

Gallup, et al. (29) fed steer calves a ration supplied with 0, 250, 500, 1000, and 2,000 ppm added Mn, as $MnSO_4$. Phosphorus excretion in the feces increased at all levels of added Mn. Calcium excretion increased at the two highest levels of Mn intake. Plasma levels of Ca and P remained unchanged.

The work of Maas, et al. (55) and Moore, et al. (73) has demonstrated a similarity between Mn and Mg uptake in plants. Maas, et al. (56) further showed that Mn and Mg had a mutually depressing

effect on the metabolic absorption of each other. At equal concentrations of these two ions in mixed-salt solutions, however, they found that Mn absorption considerably exceeded that of Mg. In further work they found that Mn absorption was stimulated by the presence of Ca. However, when Ca and Mn were in the presence of Mg the Mg inhibition of Mn was greatly accentuated. The combination of Ca and Mg apparently had a greater inactivating effect on the Mn carrier than Mg alone. The absorption of Mg in this system was also slight. Potassium absorption in this Ca-Mg-Mn system was greatly enhanced.

Manganese absorption by plants is also influenced by temperature, O_2, microorganism and root exudates in the root environment. The reducing conditions in the wet soils, and the warming trend above 14 C often noted to accompany grass tetany, would favor the reduced Mn^{+2} form that is available to plants. Mederski and Hoff (66) reported that soybeans grown at 27 C contained more than twice the Mn concentration of plants grown at 15 C.

Aluminum is present in higher plants in variable but usually low concentrations. Most edible grasses and clovers are reported to contain 10-50 ppm Al on a dry matter basis (89,93). There are certain accumulator species in the plant world that are notable exceptions. Maiden and Smith (59), in 1895, and Smith, (97) in 1903, reported massive deposits of basic aluminum succinate [$Al_2 (C_4 H_4 O_4)_3 Al_2 O_3$ - suggested composition] in a cavity of Orites excelsa R. Br. of the family Proteacea. Webb (113) detected 80 aluminum - accumulating species among 1,324 species tested from Australian - New Guinea flora. He found certain dicotyledons (69 spp.) and Filicales (11 spp.) to be the

strongest accumulators and noted that none of the local accumulators detected were on alkaline soils.

Nagistads (58) work indicated that plants absorb Al at higher pH values. Jones (45) found that plants grown in fly-ash deposits at high pH values accumulated Al in the leaf tissues but stated that it seems unlikely that Al would enter the plant as the anion in view of the fact that root surfaces have been shown to be on the acid side of neutrality. The width of this acid rizosphere zone would be influenced by the root, the root exudates and the extent and activity of the micro-organisms present. Aluminum in this zone would either percipitate as hydroxide or phosphate or be prevented from percipitation by the production of complex-forming compounds.

A major symptom of Al toxicity is exhibited as phosphate deficiency. Many plants are tolerant of high levels of Al, however, and maintain their phosphate status. Some accumulate rather than exclude Al as a direct association with their Al tolerence (28). Jones (45) stated that in order to be transported, Al "must remain in a soluble form at physiological pH values in the presence of phosphate," and suggested that organic acids chelate Al and thus prevent Al-P percipitates from forming. DeKock and Mitchell (17) have shown that Al chelated with ADA, DTPA and EDTA was absorbed by mustard plants in nutrient solution.

High levels of soluble Al usually occur in soils under acid conditions. Small (96) demonstrated that acidiphilous plants usually have strong organic acid-buffer systems in their cell sap whereas alkaliphiles generally possess phosphate buffer systems.

Rhue (83) suggested from respiration inhibitor studies with 2, 4-dinitrophenol, that Al uptake is by passive diffusion across the plasmalemma and that the part metabolism plays is in the maintenance of root membrane structure and integrity.

Aluminum ingested by animals under normal conditions has not been shown to constitute a toxic hazard. Ingested Al is very poorly absorbed and is excreted in the feces (43,110). Ondieicka, et al. (80) showed that high doses of aluminum sulfate (200 mg/kg) greatly increased retention in rats. Increased Al levels appeared in urine and body tissues, particularly the liver, testes, and bones. Apparently animals cope with moderate increases in Al intake by fecal excretion but absorption and retention are elevated by very high levels of intake.

Excessive Al intake interferes with P absorption, apparently as a result of the formation of insoluble, non-absorbable phosphate-aluminum complexes (44,80). Jones (44), in a study of rat serum, showed that chronic and acute intoxication with $AlCl_3$ resulted in a decrease in ATP and an increase in both AMP and ADP and suggested that the shift in equilibrium was possibly connected with a decrease in inorganic P levels in the blood. Aluminum has been reported to decrease hexose phosphorylation, the hexokinase-catalyzed reaction yielding sugar phosphate and ADP (9,10,87). Inhibition of glycolysis and phosphorylation appear to be the most outstanding effects of excess Al. Sorenson suggested the possibility that "all phosphate transferring systems involving ATP and Mg may be biological targets for excess Al" (98).

Little information is available on the effects of high levels of

aluminum intake in ruminants. Hobbs (40) and co-workers fed a diet containing 405 ppm Al as aluminum sulfate to cattle and sheep and observed no adverse effects. Thompson, et al. (104) fed an 810-ppm aluminum sulfate diet to sheep and observed no effect. Valdivia, et al. (111) fed steers up to 1200 ppm Al as aluminum cloride and observed no influence on animal performance and only minor changes in tissue mineral composition. They reported no effect on plasma levels of phosphorus, calcium or magnesium.

In what appears to be the only paper dealing with the subject, Dennis (18) reported finding up to 1000 ppm Al in oat (Avena sativa, L.) and wheat (Triticum aestivum, L.) tetany pastures and suggested that it may in some way be related to the occurrence of grass tetany.

Wilkinson and Stuedeman (115) reported increased forage Al concentrations in pastures receiving increased rates of N fertilizer.

MATERIALS AND METHODS

A. Case Histories

A three-year investigation was begun in February 1977 to characterize grass tetany cases. Hypomagnesemia was established by determination of blood serum magnesium levels and by clinical diagnosis by a veterinarian. Within 24 hours after tetany was reported, forage, soil, soil moisture, and root samples were collected from the pasture where the animals had been grazing. Sampling sites were selected to represent both well-drained and poorly-drained areas in each pasture. Visual evaluations were also made at each site to estimate well-drained, wet, saturated or ponded conditions. Each forage sample and the corresponding soil and root sample represented an area 92 cm's in diameter. Soil samples were taken to a depth of 8 cm. Dead tetany animals were incised and the undigested, solid material of the rumen content was sampled. In an attempt to establish approximate mineral levels present in bovines under non-tetany conditions, samples were also taken from fistulated animals grazing under various conditions at different times.

Forage and rumen content samples were dried for 24 hours at 60 C and ground in a stainless-steel Wiley mill to pass a 20-mesh sieve. One-gram samples of plant and rumen material were wet-ashed with nitric-percloric acid. The resulting residue was filtered through Whatman No. 42 and brought to a 100-ml volume with distilled water. Aluminum, Mn, Zn, K, Na, Ca and Mg were determined on appropriate dilutions by atomic absorption spectroscopy. Lanthanum oxide was included in the Ca and Mg dilutions to suppress phosphate interference. Phosphorus was determined

by the vanadate-molybdate yellow method.

Soil samples were analyzed for P, K, Ca, Mg, Na, Mn, and pH by the Louisiana State University Soil Testing Laboratory. Extracting solutions used were 0.1N HCl + 0.03N NH_4F for P, 1N NH_4OAc at pH 7.0 for K, Ca, Mg and Na and DTPA-TEA (0.005M DTPA, 0.01M $CaCl_2$ and 0.1M TEA) extracting solution for Mn. Determination of pH was made using a 1:1 soil to water ratio. Total acidity and exchangeable Al were determined by extraction with 1N KCl and titration with 0.1N NaOH and 0.098N HCl using phenolphthalein indicator and NaF to complex Al.

Soil moisture levels were determined by drying samples in an oven at 100 C for 24 hours. Percentage moisture was calculated on the over-dry basis. Three samples, each consisting of three sub samples, were taken at each site within the field. Their moisture percentages were then averaged to give the final soil moisture percentage at each sampling site.

Root samples were examined by light microscopy for the presence of endomycorrhizal fungi. Roots were washed free of soil, placed in 10% KOH for one hour at 90 C, rinsed with acetic acid and transferred into a solution of lactophenol containing 0.01% cottonblue for an additional hour at 90 C. The stain was poured off and the roots transferred to a solution of lactophenol without cottonblue. Root segments were evaluated under the microscope. The extent of mycorrhizial infection was ranked on the basis of many, several, few or no visible fungal hypha. This method was not intended as an attempt to quantitate the numbers present. Rather it was used as a means for relative comparisons of levels of infection between locations and treatments.

The above methods for analysis of plants, soils, soil moisture,

and mycorrhizial infection were used in the following experiments except where otherwise indicated.

B. Field Experiments

Field plots were established in three pastures which had a history of grass tetany, to determine the effects of various fertilizers on several forage mineral levels. Treatments consisted of a control, dolomite (2240 kg/ha), P (20 kg/ha - as treble superphosphate), Mg (60 kg/ha - as $MgSO_4$), the combined levels of P and dolomite, and the combined levels of P and Mg. A randomized block design was used and treatments were replicated four times. Locations, soils, dates of plot establishment and harvest and soil analyses at each location are given in Table 1. Ryegrass (Lolium multiflorum, Lam.) pastures were cultivated, planted and fertilized by the normal methods of the farm operation before treatments were applied. Fertilizer treatments were spread by hand. Plots were not fenced and animals had free access to them. A prolonged fall drought delayed both pasture and plot establishment. Delayed growth and animal foraging limited the collection of samples. Samples were collected at each harvest for forage analysis.

C. Greenhouse Experiments

1. Moisture Levels and Mycorrhizial Fungi

The effects of soil moisture and mycorrhizial fungi on the concentration of several minerals in ryegrass forage were investigated in a greenhouse experiment using a completely randomized design with a split plot arrangement of treatments. The soil in the experiment was a Granada silt loam, taken from the surface horizon (0-10 cm) of a cultivated field on the Sonny Guttziet farm, 12 kilometers west of Clinton,

Table 1. Locations, soil types, soil test values and dates of establishment and harvest of field plots.

Location	Soil Type	Date Established	Dates Harvested	Soil Test Values						
				P	K	Mg	Ca	Mn	Na	pH
				ppm						
Long Ranch Point Coupee Parish	Commerce silt loam (Aeric Fluvoquent)	12/22/76	3/17/77	96	190	295	1285	18	80	5.6
Beechgrove Plantation East Feliciana Parish	Calloway silt loam (Glossaquic Fragiudalfs)	12/16/76	3/1/77 3/17/77	167	159	144	830	79	52	6.2
Guddziet Farm East Feliciana Parish	Granada silt loam (Glossic Fragiudalf)	1/11/77	1/30/77 2/16/77 3/ 8/77	86	99	117	820	190	46	6.3

Louisiana. This soil is classified as a Glossic Fragiudalf and by analysis contained 133 ppm Mn, 44 ppm Na, 93 ppm Mg, 720 ppm Ca, 91 ppm K, 72 ppm P, 1.7% organic matter and had a pH of 5.7. The soil was passed through a 6 mm sieve and uniformly mixed. Pots, doubly lined with plastic bags, containing 5 kg of the air-dry soil were planted with ryegrass. The three treatments with four replications each were as follows: control, inoculation with Endogone-sporocarps (obtained from B. Mosse, Rothamsted Experimental Station), and a saturated soil condition (saturation point was calculated and maintained by weight). All pots were watered with distilled water. The control and Endogone inoculated pots received enough water so that water was not a limiting factor to growth but was not allowed to approach saturation. Pots were inoculated with Endogone at the time the seeds were planted. The excess moisture treatment was begun on day eight. Nitrogen, as ammonium nitrate, was dissolved in distilled water and applied to all pots at the rate of 50 ppm on days 10, 21, 34, and 40. Pots were rerandomized twice during the experiment. All plants were harvested by clipping to a 2-cm stubble height on days 24, 40, and 70. Samples were saved for chemical analysis. Root samples were evaluated under the microscope for the presence of endomycorrhizal fungi.

2. Moisture Levels and Soil Types

A second greenhouse experiment was designed to determine the effect of soil moisture level on mineral uptake and on extent of endomycorrhizal infection in ryegrass. Five levels of soil moisture: ponded, saturated, 100, 300, and 600 cm suction, were used on each of two soils, Mhoon silty clay loam (Typic Fluvaquent) and Falaya silt loam

(Aeric Fluventic Haplaquent). The completely randomized design with a factorial arrangement of treatments included four replications of each treatment on each soil. The Mhoon soil was taken from a surface horizon (0-10 cm) on the Long Ranch in Point Coupee Parish and the Falaya was collected from a surface horizon (0-10 cm) on Beechgrove Plantation in East Feliciana Parish. Enough soil was collected for use in several of the following experiments. The profile analyses of these soils taken at the site of collection is given in Table 2. Analyses of the collected soils are given in Table 3. The soils were brought into the greenhouse and air-dried. The Falaya soil was passed through a 6-mm sieve. The Mhoon soil was ground in a Braun Chipmunk crusher. Each soil was thoroughly mixed for representative sampling.

Three kilograms of air-dry soil were packed into pots doubly lined with plastic bags and 0.65 g of ryegrass seed were planted in each of the 40 pots. Nitrogen, as ammonium nitrate, was applied in solution at the rate of 50 ppm at the time the seeds were planted.

Soil water suction relationships were determined for four suction values on a disturbed soil sample using the pressure plate method (5). These values are shown in Table 4. Such relationships were used to maintain suction values of 100, 300, 600 cm of tension in the pots. Different amounts of water were, added at different times for the different suctions in order to maintain the various suction values. Figures 1 and 2 show the fluctuations of water suction with time for the three treatments. Such a procedure for the maintenance of the desired suctions with minimum fluctuation was considered adequate. The suction values shown in Figures 1 and 2 were determined using soil-water

Table 2. Analysis of profile samples of Falaya silt loam and Mhoon silty clay loam soils used in growth chamber and greenhouse experiments.

Soil	Sample Depth	P	K	Ca	Mg	Na	Mn	Exchangeable Al	Total Acidity	pH
	cm	ppm						meq/100g		
Falaya	0-5	244	155	1100	170	102	84	0.00	0.4	5.8
	5-13	129	69	1195	162	84	127	0.00	0.3	6.0
	13-23	33	60	1070	148	90	127	0.00	0.2	6.1
	23-36	31	53	870	128	113	59	0.00	0.3	5.4
	36-71	29	44	370	96	104	15	2.06	3.6	4.9
	71-106	29	48	240	163	181	5	4.80	4.9	5.0
Mhoon	0-8	182	130	4000+	673	103	12	0.00	0.2	7.3
	8-15	158	109	4000+	670	114	9	0.00	0.2	7.4
	15-41	122	123	4000+	717	113	9	0.00	0.2	7.4
	41-58	206	83	3415	599	109	10	0.00	0.2	7.4
	58-74	234	100	3700	666	102	11	0.00	0.2	7.4
	74-102	277	78	3225	563	96	7	0.00	0.2	7.6

Table 3. Analysis of the Falaya silt loam and Mhoon silty clay loam surface (0-10 cm) soils used in the growth chamber and greenhouse experiments.

Soil	P	K	Ca	Mg	Na	Mn	Exchangeable Al	Total Acidity	pH
	ppm						meq/100g		
Falaya	225	199	1195	232	87	104	0.0	0.4	5.9
Mhoon	196	174	4000	732	88	9	0.0	0.3	6.7

Table 4. Soil water content* of Falaya silt loam and Mhoon silty clay loam determined by the pressure plate method at four suction values.

0.1 bar		0.3 bar		0.5 bar		1.0 bar	
Mhoon	Falaya	Mhoon	Falaya	Mhoon	Falays	Mhoon	Falaya
g/100g							
34.86	36.77	29.51	30.05	26.51	26.59	24.48	23.51

* Average of four replications

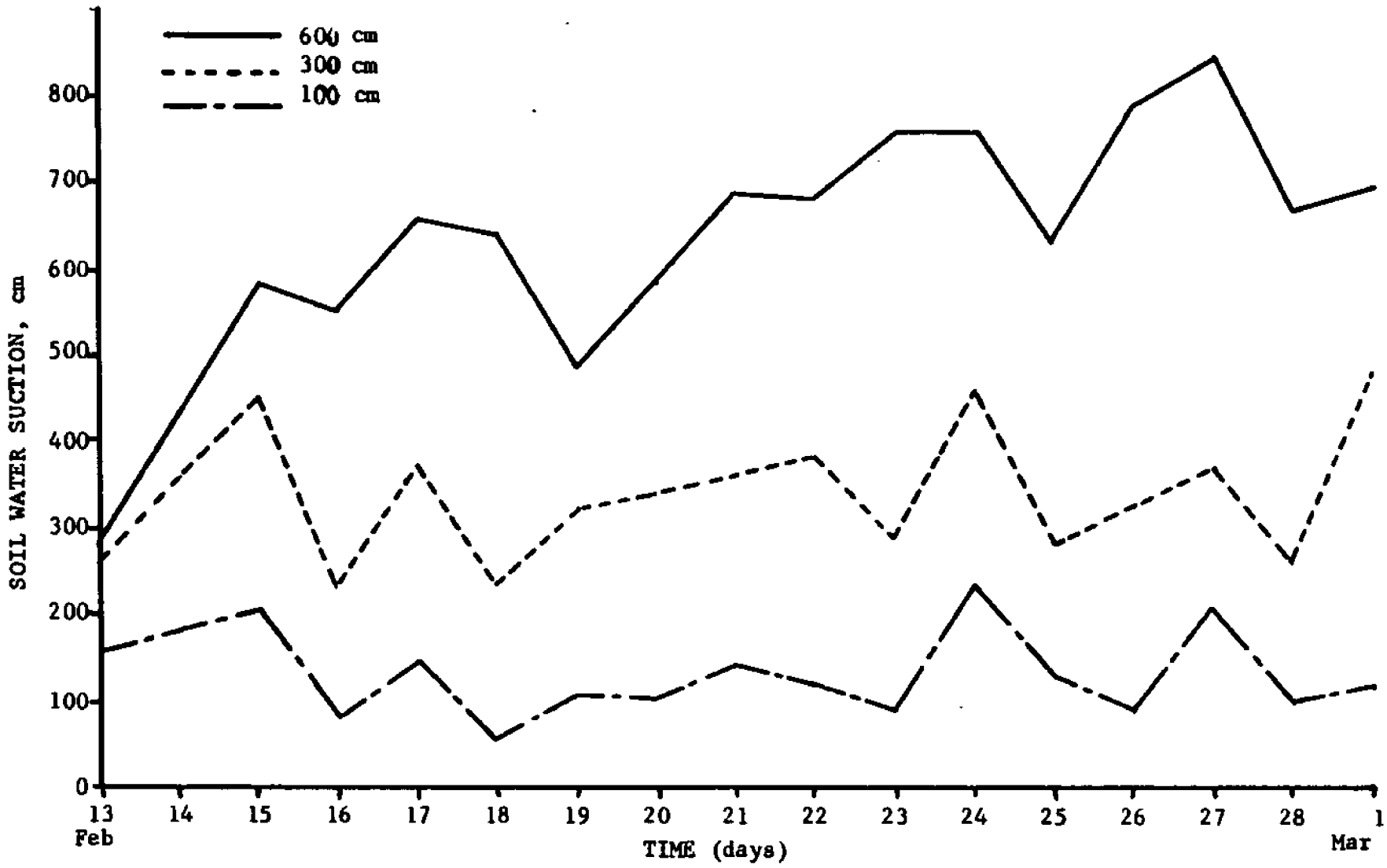

Fig. 1. Daily fluctuations in soil water suction values obtained for 100, 300, and 600 cm treatments in Falaya silt loam.

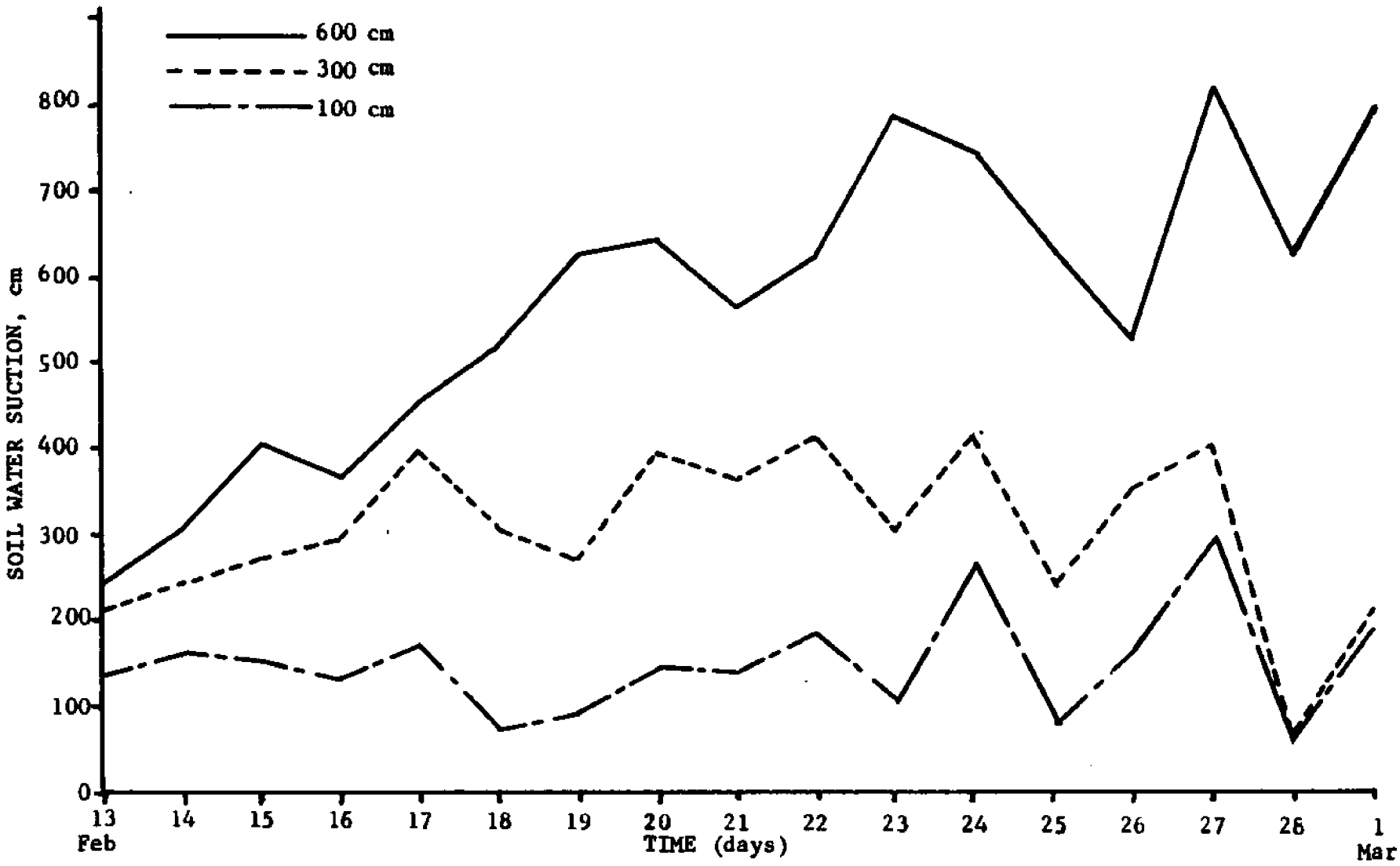

Fig. 2. Daily fluctuations in soil water suction values obtained for 100, 300, and 600 cm treatments in Mhoon silty clay loam.

tensiometers (2.22 cm diameter porous ceramic cups) connected to a mercury monometer (5). One tensiometer was installed in each pot for the three treatments during soil packing. Tensiometer readings were recorded at least twice a day and different volumes of water were accordingly added in order to maintain the desired suction values.

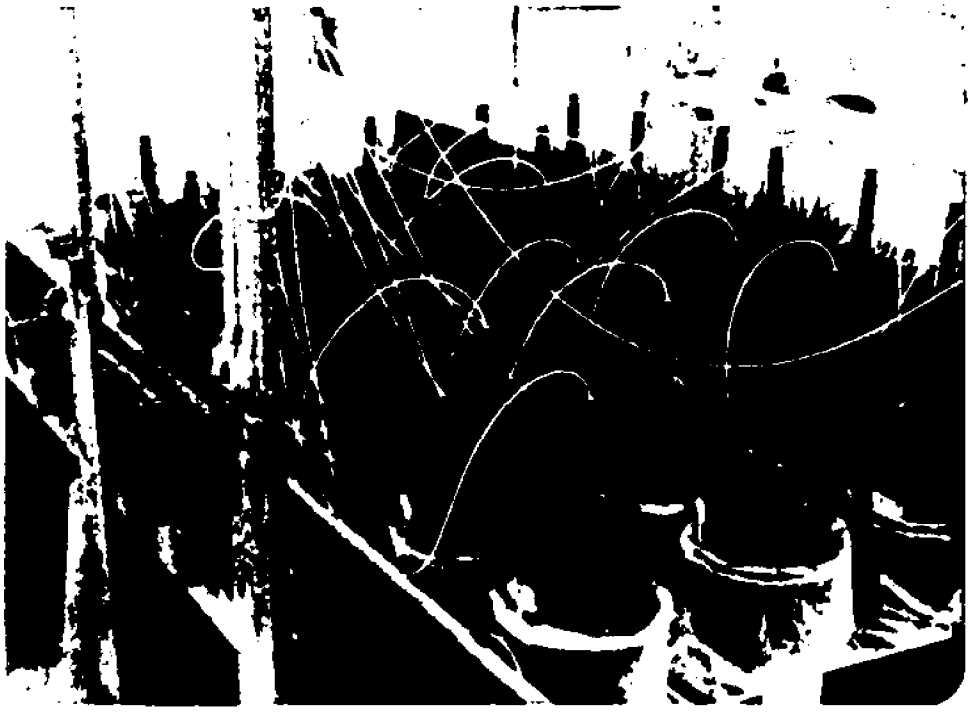

Figure 3. Soil moisture levels monitored with tensiometers connected to mercury monometers in the greenhouse experiment.

The ponded and saturated treatments were maintained by visual evaluation. Approximately one-half inch of water above the level of the soil was maintained for the ponded treatment. The saturated soils were given sufficient water to keep the water level at the soil surface.

At the termination of the experiment at the end of 26 days the grass was harvested and the tensiometers were disconnected. Plant samples were saved for chemical analysis. The water in the porous cups

was emptied using a hand vacuum pump. Additional tensiometers were installed in the ponded and saturated pots after the grass was harvested. The tensiometer cups were then used as a soil solution sampler in order to obtain a sub-sample of the soil solution for the analysis of Al, Mn, Zn, Mg, Ca, P, and K. This was achieved by applying a vacuum of "0.8 bars" for approximately 24 hours. Soil solution samples were easily obtained, frequently within two hours, for the low suction treatments. As much as 24 hours were needed to obtain a soil solution sample for the high suction treatment.

Root samples were taken at the time of harvest from each pot and evaluated for mycorrhizal fungi.

Samples of soil were taken from each pot for moisture determination. One sample was taken from the surface soil (0.5 cm) and a second sample was taken below the 5-cm depth. Each sample consisted of two sub-samples. Only surface samples were collected from the ponded and saturated treatments.

D. Growthchamber Experiments

1. Temperature and Soil Types.

A three-part growth chamber experiment was conducted in order to study the effects of temperature on mineral levels in ryegrass forage. A completely randomized design with a split-plot arrangement of treatments was used and included one level of soil moisture, two soils and five harvest dates. The two soils were the Falaya silt loam and Mhoon silty clay loam described previously. Plastic flats, each containing 15 kg of air-dry soil, were prepared. Each soil was replicated four times, giving a total of eight flats. Five parallel rows of ryegrass seed

were planted in each flat and nitrogen, as ammonium nitrate, was applied at the rate of 50 ppm on day one of the experiment. In trial one, the flats were placed in a greenhouse for a period of 17 days. Greenhouse temperatures ranged between 18 and 30 C. On day 18 the flats were placed in a growth chamber where they remained for 22 days. Air temperatures were maintained in a range of 8-11 C and soil temperatures were held at 7-11 C. The growth chamber, especially designed and built for this project provided 2200-2800 foot candles of light by means of eight 60-watt incandescent bulbs and ten 120-watt cool white, 96 inch, very high output fluorescent bulbs. The growth chamber was placed in a controlled temperature room. Plants were exposed to alternating periods of light and dark of 12 hours duration each. By day 21 all soils had been brought to a saturated moisture condition with distilled water. The saturation point was maintained for the remainder of the experiment. On day 39, after 22 days of cold temperature exposure, the flats were returned to the greenhouse where air temperatures of 28-33 C and soil temperatures of 21-25 C were maintained for the remaining eight days of the experiment. One row of grass taken at random from each flat was harvested on day 39 before flats were transferred to the greenhouse. The remaining four rows were randomly harvested from each flat on alternate days thereafter. The experiment was terminated after the fifth row was harvested on day 47. Ryegrass was harvested at a two-cm stubble height, dried, ground and chemically analyzed.

Trial two was repeated in the same manner except that the cold temperatures of the growth chamber were lower. Air temperatures of 2-5 C and soil temperatures of 0-3 C were maintained for the 22-day period. An

electric fan was installed to circulate air and assist in maintaining the cold temperature by moving heat away from the lights. On the 12th day of the cold treatment a breakdown in the system allowed the temperature to rise to 35 C for several hours. Low temperature was reestablished however within 18 hours. Flats were rerandomized one additional time while they remained in the growth chamber. Trial three was conducted in the same manner with air temperatures during the 22-day cold period ranging from 8-13 C and soil temperatures of 8-12 C. Greenhouse temperatures during the final eight days of the third trial were slightly higher than before, ranging from 28-34 C and 24-27 C for air and soil temperatures, respectively.

2. Temperature, Soil Type and Moisture Level

A second growth chamber experiment was designed to include two soils, two levels of soil moisture and five sampling dates. The completely randomized design with a split-plot arrangement of treatments was conducted as the previously described growth chamber experiments with the following exceptions: the entire experiment was conducted inside the growth chamber with the warm periods maintained at 75 $\pm$4 C and the cold period maintained at 1-3 C air and 0-1 C soil temperatures; flats were rerandomized daily; and cold treatment was continued for 16 instead of 22 days due to loss of control of the cooling system. Soil moisture levels were maintained at 0.3 bars and a ponded condition by the methods described in the greenhouse experiment. The ponded condition was established by day six of the experiment. Harvesting of forage samples was begun on the last day of cold treatment and continued on alternate days through the five sampling dates. Soil solution samples

were taken from the ponded soils at the time of each harvest through tensiometers. Solutions were filtered through No. 42 Whatman filter paper. Aluminum, Mn, Ca, Mg and K were determined directly on the filtrate, with no further dilutions or additions. Phosphorus was determined by the ammonium molybdate-sulfuric acid stannous chloride method. Forage samples were analyzed as previously described.

E. Bickham Pasture Investigation

Field plots were established in a pasture on the L. Bickham farm, approximately 16 kilometers south of St. Francisville, Louisiana, in order to monitor forage levels of Al, Mn, Zn, Mg, Ca, P, and K from the time of pasture establishment through the grass tetany season. Four locations were selected to include both well-drained and poorly-drained sites. A three-meter square metrics plot was staked and fenced at each location after the ryegrass pasture was established by the normal practices of the farmer. Soil temperature was monitored by means of thermometers inserted in the soil to a depth of 8 cm at each location. Readings were taken at the time of forage sampling. Air temperature was constantly monitored by a Weather Measure Model H311 hygrothermograph. Soil moisture was also determined at each location each time forage samples were collected. Determinations were made by oven-drying soil samples at 100 C for 24 hours. Three samples, each consisting of three sub-samples taken to a depth of 8 cm, were collected at each site. The percent moisture was determined on each of the three samples and then averaged to give the percent moisture at each location. A visual determination of soil moisture was also recorded on the basis of ponded, saturated or less then saturation conditions.

Forage samples for chemical analysis were collected randomly within each plot every four to seven days beginning October 29, 1978 and continuing through April 1, 1979.

Locations A and B were on Loring silt loam soil (Typic Fragiudalf) and location C was on Memphis silt loam (Typic Hapludalf). These were nearly level to gently sloping, well-drained, moderately permeable upland soils formed in loess more than four feet thick. Location D was on Falaya silt loam (Aeric Fluventic Haplaquent), a somewhat poorly-drained alluvial soil derived from silty alluvium from loess. The soil profile analyses are in Table 5.

F. In Vitro Experiments

An in-vitro study was designed to determine the effects of Al and Mn on Mg and Ca solubility. Half-gram (0.5 g) samples of dried, ground ryegrass and 25 ml of prepared buffer solution (McDougall's artificial saliva buffer) were placed into each of 14 centrifuge tubes. Seven treatments were used in duplicate as follows: Control, 1,000 and 2,000 ppm Mn, 4,000 and 8,000 ppm Al, 1,000 ppm Mn plus 4,000 ppm Al, and 2,000 ppm Mn plus 8,000 ppm Al. Treatment concentrations are expressed on the basis of ppm in dry plant material. Treatment concentrations in the total 37-ml final volumes were 14 and 28 ppm Mn, 57 and 114 ppm Al and the respective combinations of the low and high treatments. Each element was added in the sulfate form. Treatments were added by preparing six stock solutions, one for each treatment level of Mn and/or Al, so that the 2-ml aliquot of stock solution contained the desired treatment concentration. Two milliliters of distilled water was added to the controls. The tubes were placed into a water bath

Table 5. Analysis of profile samples taken at sites A, B, C, and D in the Bickham research pasture.

Site	Sample Depth	P	K	Ca	Mg	Na	Mn	Exchangeable Al	Total Acidity	pH
	cm	ppm						meq/100g		
					Loring silt loam					
	0-10	63	96	1108	280	43	64.9	0.00	0.30	7.2
A	10-20	6	39	617	134	41	104.0	0.00	0.20	6.5
	20-36	5	37	812	163	46	37.7	0.00	0.20	6.2
	36-61	5	90	1477	431	73	12.4	0.00	0.25	6.2
	61-86	12	79	1037	468	116	11.4	0.49	1.10	5.6
	86+	16	65	685	393	102	17.1	1.57	2.90	5.5
					Loring silt loam					
	0- 8	42	57	1678	91	46	72.5	0.00	0.30	7.1
B	8-18	9	53	664	89	41	147.9	0.00	0.30	5.9
	18-38	5	26	578	151	53	98.6	0.20	0.50	5.6
	38-56	5	70	858	441	107	24.4	0.59	1.60	5.6
	56-86	8	64	721	414	104	19.4	1.62	2.60	5.5
					Memphis silt loam					
	0- 6	19	37	926	99	50	95.3	0.00	0.30	6.3
C	6-25	5	19	398	86	48	114.4	0.49	1.00	5.5
	25-41	5	22	446	175	65	58.7	0.24	0.80	5.7
	41-81	5	66	484	349	92	12.3	2.65	3.20	5.5
	81-107	5	96	349	358	104	8.3	3.23	4.80	5.4
					Falaya silt loam					
	0- 5	91	91	1852	154	67	50.8	0.00	0.30	6.9
D	5-18	20	48	868	120	61	74.2	0.00	0.30	5.9
	18-56	6	16	400	94	68	89.3	0.59	1.20	5.5
	56-81	5	21	377	141	81	37.3	0.98	1.50	5.6
	81+	5	61	443	323	141	17.8	4.65	6.45	5.4

(39 C) and allowed to stand for 15 minutes. Each tube was then inoculated with 10 ml of rumen fluid. Tubes were flushed with CO_2 for 15 seconds and stoppered tightly. The final volume in each tube was 37 ml.

At the same time, a second set of tubes was prepared exactly as the first except that they contained no forage. A third set was simultaneously prepared leaving out both forage and rumen fluid so that they contained only buffer and treatment solutions. Ten milliliters of distilled water were added to each tube in the third set to maintain the same final volume and treatment dilution rates. All tubes were incubated with constant agitation for 48 hours. Tubes of set one and two received a constant flow of CO_2.

After 48 hours the tubes were removed from the water bath, placed in a centrifuge for 10 minutes at 1800 g and filtered through Whatman No. 41 filter paper. Magnesium and Ca were determined on the filtrate, with no further dilution.

G. In Vivo Experiments

1. Fistulated Steers

Four fistulated steers were treated with Al and Mn in the sulfate form, in order to study their effects on blood serum Mg and Ca levels in vivo. One Holstein and three Hereford Steers were placed in a Latin Square experiment. All animals were maintained on a basal diet of bermuda grass hay which analyzed as follows: Mn 220 ppm, Al 40 ppm, Mg 2850 ppm, Ca 2800 ppm and K 1.8%. Animals were individually stalled, fed, and watered. The Herefords received 7 kg and the Holstein 9 kg of hay daily which was normally totally consumed. Water was supplied free choice. No other minerals or supplements were fed. All animals

were standardized to the hay diet for 19 days before treatment began. Treatments were as follows: Control, 4,000 ppm Al, 2,000 ppm Mn, and 2,000 ppm Mn plus 4,000 ppm Al, calculated as ppm of dry daily feed allotment. Aluminum and Mn were dissolved in 2,000 and 500 ml of distilled water, respectively and were administered via rumen cannula into the ventral sac. Animals were initially selected at random for treatments but during successive treatment periods treatments were rotated until every animal had been on every treatment. Blood and rumen fluid samples were collected, treatments administered and animals fed at the same time each morning.

Blood samples were drawn by vena puncture of the jugular vein using a 20-gauge needle and a 15-ml vacutainer collection tube. The samples were allowed to clot for three hours at room temperature then centrifuged for 25 minutes at 4,600 g. Two-milliliter aliquots of serum were pipetted into clean vacutainers. Twenty milliliters of glass-lined distilled-water containing 5% HCl was added for a 1:11 dilution rate. Calcium and Mg were determined with an atomic absorption spectrophotometer with no further dilution or additions.

Rumen fluid samples were obtained and immediately strained through eight layers of cheese cloth into plastic bags. Samples were then taken into the laboratory, centrifuged for 10 minutes at 1800 g and filtered through No. 41 Whatman filter paper. Aluminum and Mn were determined on the filtrates. A five-ml aliquot was diluted with distilled water to give a 1:10 dilution for the determination of Ca and Mg.

A second sample of rumen fluid was taken from each animal for an immediate determination of pH using a Beckman Chem-Mate Model 73 pH

meter. Collection of blood and rumen fluid samples began at lease four days before treatments began and continued after the end of the four day treatment period until serum Mg levels were back within normal limits.

2. Lactating Cows

Eight crossbred lactating cows, approximately two weeks post-partum, were randomly divided into three treatment groups in a completely randomized design with a split-plot arrangement of treatments. All animals were maintained on bermuda grass hay and were group-fed free choice under drylot conditions with free access to water. The hay contained 190 ppm Mn, 100 ppm Al, 2050 ppm Mg, 3500 ppm Ca, 1200 ppm P and 1.45% K. Animals were standardized to the hay diet for six days before treatments began. Group one, the control group, contained two animals. Groups two and three, with three animals each, were dosed once daily with 4,000 ppm Al and 4,000 ppm Al plus 2,000 ppm Mn, respectively, calculated as ppm in a 9 kg average daily feed intake. Both Al and Mn were in the sulfate form. Aluminum and Mn dissolved in 2000, in 500 ml of distilled water respectively, were administered by stomach tube. Treatments were continued for a total of four days. Blood samples were collected each day at the time treatments were administered and handled in the same manner as in the previous experiment. Collection of blood samples began five days before treatments began and continued for four days after treatments were discontinued. Rumen samples were not collected in this experiment.

RESULTS AND DISCUSSION

A. Case Histories

Grass tetany was diagnosed at eleven different locations in six parishes in Louisiana and one location in Tennessee during the three years of this investigation. Tetany occurred on several types of pasture forages including ryegrass, ryegrass and oats, ryegrass and wheat and fescue (Festuca arundinacea, Schreb.). One grass tetany outbreak was also diagnosed in a group of dairy cows, closely following parturition, on a diet of ryegrass hay supplemented with a concentrate ration. Several animals died after hay feeding began. Mineral analyses of forages from tetany pastures are given in Table 6. Although three to twelve forage samples were collected from each pasture, the mineral analyses are reported here for only the samples with the highest and lowest Al levels.

The data in Table 6 indicate that the Al levels found in tetany field forages were much greater than the levels normally expected to occur in agronomic crops. Underwood (110) in a review of the literature reported 10 to 50 ppm Al as being typical of most grasses and clovers. Aluminum levels in the tetany pastures of this study commonly occurred in the 2,000- to 8,000-ppm range with one sample containing 14,500 ppm Al.

A second important point obtained from the data in Table 6 was that variability in forage Al concentrations among sampling sites within pastures was very great. The sample with the highest Al level in each pasture generally contained two to eight times higher concentrations than

Table 6. Mineral analyses of forage samples from grass tetany pastures in Louisiana, 1977-79.

Pasture No.	Sample+ No.	Date	Al	Mn	Zn	Ca	Mg	P	K
			ppm						%
1	1	2/3/77	4140	240	39	4650	1800	3100	2.65
	2		3050	270	33	4150	1500	2850	2.85
2	1	2/5/77	2200	1840	34	1400	1050	3100	2.60
	2		980	380	26	2300	1100	2700	2.40
3	1	2/6/77	1730	120	22	3550	1100	2050	2.80
	2		720	170	16	2850	1050	1900	3.00
4	1	2/9/77	1620	120	26	2450	1450	2700	3.40
	2		840	110	25	2850	1300	2900	2.60
5	1	3/25/77	1250	180	28	6250	2350	4450	4.90
	2		250	190	38	4150	3100	4200	4.55
6	1	1/24/78	450	382	23	4875	1800	2700	1.83
	2		.60	243	27	3500	1950	2850	2.32
7	1	2/9/78	5960	135	54	4750	2450	3050	1.84
	2		720	84	37	5500	1350	2750	1.51
8	1	2/15/78	6170	248	35	5250	1750	2000	1.44
	2		3730	522	32	4500	1400	2650	1.68
9	1	2/19/78	6780	242	34	3750	1850	2900	2.03
	2		1840	396	35	5950	1550	2950	1.58
10	1	2/23/78	4410	741	30	3875	1600	2850	2.34
	2		1620	665	26	3650	1200	3050	2.50
11	1	1/25/79	14500	140	68	3600	3350	4450	2.70
	2		390	80	45	1700	1600	4650	3.40
12‡	1	6/79	8020	180	49	5900	2025	2600	1.45
	2		90	340	45	6200	1925	2800	1.34

+ Sample no. 1 and 2 represent samples with the highest and lowest Al values, respectively, found in each pasture.

‡ Forage at this location was fed as hay rather than grazed pasture.

the sample with the lowest Al level. These differences in Al levels were even greater in pastures numbers 11 and 12. It was found that Al levels in the field frequently changed drastically within a short distance. As a result, high levels of Al accumulation in the forage occurred in spots rather than being evenly distributed in the pasture. It would be entirely possible for individual animals in such a field to accumulate very different levels of Al in the rumen, depending upon where grazing took place. Areas of high Al accumulation appeared to be as closely grazed as areas with low Al levels. If there was an animal preference for forages with either high or low levels of Al, it was not detected.

Relatively high Mn concentrations were found in some samples as indicated in Table 6. Blakemore (6) considered 540-1320 ppm Mn to be high for ruminant diets and suggested a possible association with grass tetany. However, the forage Mn levels in this study were not consistently high. Only five of the twelve locations were found to contain forages which exceeded 500 ppm Mn. A low correlation was found under grass tetany conditions between the accumulation of Al and Mn (Table 7).

Concentrations of Mg were generally below 0.20%, a result that other researchers have frequently noted. However, a significant positive correlation was found to exist ($p \leq 0.001$) between Mg and Al accumulations in forages from the tetany fields (Table 7). The observed levels of Zn, Ca, P, and K were typical of many cool-season forage crops. Potassium was significantly correlated to P accumulation ($p \leq 0.001$). Unusually high K concentrations were observed at only one location. A

Table 7. The correlation coefficients between soil moisture levels and mineral concentrations in forages from grass tetany pastures, 1978 and 1979.

Variable	Al	Mn	Ca	Mg	P	K
	correlation coefficient					
Percent soil moisture	0.62***	0.45**	0.14	0.46***	-0.32*	0.06
Mn	0.08	--	--	--	--	--
Ca	0.14	-0.44**	--	--	--	--
Mg	0.82***	0.02	0.22	--	--	--
P	-0.30*	-0.17	-0.10	0.03	--	--
K	-0.21	0.10	-0.04	0.09	0.64***	--

(*, **, ***, denote significance at the 5, 1 and 0.1% level, respectively.)

significant negative correlation was found between P and Al accumulation ($P \leq 0.05$). No significant correlations were observed between Ca or K and Al ($P \leq 0.05$).

Soil moisture percentage was found to be highly correlated with Al accumulation ($P \leq 0.001$). A total of 58 forage samples with corresponding soil moisture samples from tetany pastures were analyzed in these correlations (Table 7). All sites with high forage Al accumulation were observed to be under conditions of high soil moisture (Table 8).

The other information obtained from analysis of the soil samples from tetany pastures was not particularly helpful in explaining the mineral levels found in the forages (Table 8). None of the soil samples analyzed contained measurable amounts of exchangeable Al. The soil pH values, at the locations of highest forage Al levels, ranged from 5.1 to 7.3 with an even distribution of values across the range. Forage Al accumulation was not closely associated with the pH of the corresponding soil sample. Extractable Mn levels were highly variable, both within and among pastures. Values ranged from 12 to 308 ppm Mn. Results obtained for the other mineral elements, including Mg, were generally within acceptable ranges.

No recurring pattern of a particular soil series was found among the twelve locations in this study. The site of highest Al accumulation occurred on eight different soil series (Table 9). They generally tended to be the more poorly-drained soils of the field, however.

Scanning root samples for mycorrhizal fungi provided no basis for comparisons among tetany fields. Mycorrhizal fungi were found to be present in most samples but at generally low levels of infection. Samples

Table 8. Analysis of soil samples taken from grass tetany pastures at the site of highest forage aluminum accumulation.

Pasture No.	Exchangeable Al	Mn	P	K	Mg	Ca	Na	pH	Moisture
	meq/100g	ppm							- % -
1	-*	79	31	88	153	1220	57	7.0	Ponded
2	-	308	115	118	26	110	67	5.1	Saturated
3	-	37	24	173	203	1095	74	6.1	Saturated
4	-	28	186	174	271	1185	72	5.2	Saturated
5	-	28	86	159	171	1145	45	6.3	Saturated
6	0	99	41	41	38	630	61	6.2	32
7	0	12	330	382	940	4000+	61	5.7	64 (Ponded)
8	0	48	48	120	175	2170	62	7.3	41
9	0	52	57	280	277	2040	61	6.6	36
10	0	101	33	94	233	990	72	5.6	45 (Ponded)
11	0	55	300	500+	755	3950	108	6.8	80 (Ponded)
12**	-	-	-	-	-	-	-	-	-

* Blanks indicate soil test data were not available.

**Animals at this location were on a hay diet.

Table 9. The forage species, soil series at the site of highest Al accumulation and geographic location of grass tetany pastures.

Pasture No.	Forage Species	Soil	Location
1	ryegrass	Memphis silt loam (Typic Hapludalf)	West Feliciana Parish
2	ryegrass	Olivier silt loam (Aquic Fragiudalf)	Zachary East Baton Rouge Parish
3	ryegrass	Loring silt loam (Typic Fragiudalf)	West Feliciana Parish
4	ryegrass	Mhoon silty clay (Fluventic Haplaquent)	Livonia Point Coupee Parish
5	ryegrass	Waverly silt loam (Fluventic Haplaquent)	Beechgrove East Feliciana Parish
6	oat and ryegrass	Bude silt loam (Glossaquic Fragiudalf)	St. Helena Parish
7	ryegrass	Mhoon silty clay loam (Fluventic Haplaquent)	West Baton Rouge Parish
8	ryegrass	Loring silt loam (Typic Fragiudalf)	West Feliciana Parish
9	ryegrass	Olivier silt loam (Aquic Fragiudalf)	West Feliciana Parish
10	ryegrass	Falaya silt loam (Aeric Fluventic Haplaquent)	West Feliciana Parish
11	wheat and ryegrass	Sharkey clay (Vertic Haplaquent)	East Baton Rouge Parish
12	ryegrass hay	--	East Baton Rouge Parish

from wetter locations generally contained fewer hypha than samples from better drained areas.

Mineral analyses of rumen content samples from tetany and non-tetany animals are given in Table 10. Non-tetany animals grazed on a variety of ryegrass and mixed grass pastures with the exception of animal A which was on a diet of alfalfa hay.

The most obvious difference in the mineral content of rumen samples from tetany and non-tetany animals is the higher Al levels in the tetany animals. Aluminum concentrations in the rumen samples ranged from 1630 to 3390 ppm for the tetany animals and from 330 to 510 ppm for the non-tetany animals. Thus, there was no overlapping of Al levels between the two groups of animals. This result was not obtained for any other mineral under investigation. Manganese levels in tetany animals were generally higher than in non-tetany animals, although the magnitude of the difference was less than it was for Al. Analysis of other minerals showed no appreciable difference between the tetany and non-tetany animals.

B. Field Experiments

Field plot experiments with various fertilizers provided little information concerning their effects on mineral concentrations (Table 11). The fall drought and delayed plot establishment were not conducive to reliable results in this experiment.

A depression of Al accumulation by Mg application at the Long location was the only significant indication of a fertilizer effect on forage Al concentration and this was only indicated by Duncans New Multiple Range Test. The F test in the ANOV was not significant. No

Table 10. Mineral analysis of rumen content samples from grass tetany and non-tetany animals in Louisiana, 1977-79.

Animal No.	Date	Al	Mn	Zn	Ca	Mg	P	K
		ppm						- % -
		Tetany animals						
1	2/3/77	2570	420	64	4900	540	5250	0.235
2	2/21/78	2350	390	62	4000	565	4900	0.470
3	2/22/78	3390	425	60	4850	1090	5700	0.880
4	2/24/78	2360	442	76	5300	820	8200	0.895
5	1/19/79	1940	50	52	5550	910	3900	0.620
6*	1/79	1630	110	49	6050	700	3500	0.600
Mean		2373	306	60	5108	771	5242	0.617
		Non-tetany animals						
A	12/77	510	65	33	16700	1445	6400	0.865
B	10/9/78	330	120	49	5700	780	5400	0.575
C	10/31/78	440	40	56	4750	680	4100	0.450
D	11/28/78	410	20	72	2650	330	4900	0.355
E	12/26/78	420	170	52	2500	655	3850	0.560
F	12/26/78	390	170	53	1750	415	2900	0.835
G	12/26/78	330	280	50	2500	710	4400	0.430
H	12/26/78	410	250	51	2650	785	4600	0.370
Mean		405	139	52	4900	725	4569	0.555

*Sample from Tennessee

Table 11. The effects of P and two Mg sources on the mineral concentrations in ryegrass forage of three locations in Louisiana, 1977.

Fertilizer	Al	Mn	Zn	Na	Ca	Mg	P	K
	ppm							%
Long Ranch								
control	105+	135	26	1010	4850	2025	3975	3.6
P	85	135	23	620	4612	1937	4925*	4.5
dolomite	90	102	22*	725	4725	2150	3925	4.2
dolomite + P	95	107	24	855	4812	2100	4437	4.4
Mg	77*	125	22*	820	4387	2087	3875	3.9
Mg + P	105	132	24	625	4975	2225*	4962*	3.5
Beechgrove Plantation								
control	166	111	20	530	4212	2000	4487	3.3
P	177	121	23	505	4342	1985	5000*	3.7
dolomite	157	72	21	366	4200	2006	4556	3.7
dolomite + P	176	82	19	482	4243	2025	4793	3.4
Mg	177	117	21	458	3793	2162*	4825	4.0
Mg + P	171	100	21	777	4187	2137	4862	4.2
Guddziet Farm								
control	217	168	18	2230	4033	1554	2566	2.4
P	200	155	17	2475	3820	1408	3337*	2.8
dolomite	200	157	17	2610	4545	1908	2737	2.5
dolomite + P	194	130	15	1968	4091	1654	3200*	2.4
Mg	225	176	19	2251	3441	2008*	2720	2.7
Mg + P	190	153	17	1736	3433	1862	2308*	2.5

\+ Means averaged over all harvest dates.
* Denotes significant difference from control ($P \leq 0.05$) according to Duncan's New Multiple Range Test.

effects of fertilizers were seen on Mn, Na, Ca, or K forage concentrations. As would be expected, forage P concentrations were increased by P fertilizer ($p \leq 0.05$). Adding P with Mg significantly increased forage P concentration at two of the three locations. Adding P with dolomite effectively increased forage P concentration at only one location. Fertilizer Mg plus P at Long Ranch location and Mg alone at the Beechgrove and Guddziet locations significantly raised forage Mg levels. The effects of the other fertilizers and fertilizer combinations on forage Mg levels were not significant. Phosphorus treatments, however, although not significant produced forage Mg levels that were consistently lower than the controls.

It is of interest to note that while these three fields had a history of grass tetany, no cases occurred in them during the course of this research although cattle grazed them continually throughout the tetany season. Aluminum levels in the field plot forage samples were mostly in the range of 100-300 ppm. The highest Al level found in any single sample was 470 ppm at the Guddziet location.

C. Greenhouse Experiments

1. Moisture Levels and Mycorrhizal Fungi

The effects of mycorrhizal fungi inoculation and two levels of soil moisture on mineral concentrations in ryegrass forage grown in the greenhouse are given in Table 12. Plants grown on saturated soil were found to contain higher concentrations of Mn and P and lower concentrations of Na, Ca, Mg, and K than plants grown under optimum soil water conditions. No effect of soil moisture was observed on Al or Zn concentrations. The effect of soil moisture on Mg level is in agreement

Table 12. The effects of mycorrhizal fungi, soil moisture, and harvest date on mineral concentrations* in ryegrass in a greenhouse.

Treatment+	Al	Mn	Zn	Na	Ca	Mg	P	K
	ppm							%
Control	89a+++	382a	53a	1135a	10704a	4787a	2262a	5.4a
Mycorr. Fungi	82a	375a	53a	1043a	11120a	4908a	2204a	5.2a
Saturated soil	70a	692a	55a	762b	7462b	2675b	2566b	3.5b
Harvest date++								
1	48a+++	227a	50a	633a	6637a	3491a	2608a	5.9a
2	95b	430b	53a	1112b	8883b	4091b	2487a	5.1b
3	98b	790c	60b	1195b	13766c	4787c	1937b	3.0c

* Average of four replications.

\+ Treatment means averaged over three harvest dates.

++ Time of harvest means averaged over three treatments.

+++ Means within the same columns followed by the same letter do not differ at the 5% level of probability according to Duncan's New Multiple Range Test.

with the work of Elkins and Hoveland (20) and Elkins, et al. (21). Plants grown on saturated soils in the experiment contained approximately half as much Mg as the control and mycorrhizal-infected plants. The effect on Ca concentrations was similar though not quite as pronounced. No differences in element concentrations were found between control and mycorrhizal inoculated plants. Microscopic examination of the roots revealed extensive infection in the roots of both groups. If the roots of the inoculated plants were indeed infected with the inoculated strain, the native strains in the control group were just as effective in influencing mineral uptake. Root samples from plants on saturated soils contained very few or no fungal hypha.

Concentrations of Al, Mn, Zn, Na, Ca, and Mg in forages were found to be highest at the time of the third harvest. These plants were found to contain the lowest levels of P and K, however ($p \leq 0.05$).

2. Moisture Levels and Soil Types

The results of an expanded greenhouse experiment to determine the effects of five soil moisture levels and two soil types on mineral concentrations in ryegrass forage are presented in Table 13. Forage concentrations of all minerals under investigation were found to be significantly affected by soil moisture ($p \leq 0.05$). Soil type effects were also found to influence the forage mineral concentrations for all minerals except Mg ($p \leq 0.05$) (Table 14). Plants grown in Falaya silt loam with an exchangeable soil Mg level of 232 ppm, contained just as much Mg as plants grown in Mhoon silty clay loam with an exchangeable soil level of 732 ppm Mg. Ponded soils produced forage which contained the greatest amount of Al and the least amount of Mg. As the level of

Table 13. The effects of five levels of soil moisture on mineral concentrations[+] in ryegrass grown in the greenhouse on Falaya silt loam and Mhoon silty clay loam.

Treatment	Al	Mn	Zn	Na	Ca	Mg	P	K
	ppm							%
ponded	151a*	263a	48a	896a	5093ab	2231a	3556a	2.7a
saturated	82b	149b	61b	1152a	4659a	2375a	4568b	3.5b
100 cm	78b	161b	69bc	1906b	5728cb	3800b	4943b	5.6c
300 cm	66b	140b	74c	1828b	6059c	3962b	4637b	5.8c
600 cm	52b	160b	74c	2031b	6371c	4012b	3212a	5.5c

* Means in the same columns followed by the same letter do not differ at the 5% level of probability according to Duncan's New Multiple Range Test.

+ Treatment means averaged over four replications and two soils.

Table 14. Mineral analysis* of ryegrass grown on Falaya silt loam and Mhoon silty clay loam and soil analysis of soil types in the greenhouse experiment with five moisture conditions.

Forage Analysis

Soil	Al	Mn	Zn	Na	Ca	Mg	P	K
	meq/100g	ppm						%
Falaya	105a++	316a	62a	912a	5348a	3260a	4515a	4.3a
Mhoon	67b	34b	69b	2213b	5816b	3292a	3852b	5.0b

++ Means within columns followed by the same letter differ significantly at the 5% level according to Duncan's New Multiple Range Test.

Soil Analysis

Soil	meg/100g	ppm						
Falaya	0	104	--	87	1195	232	225	199
Mhoon	0	10	--	88	4000+	732	196	174

* Treatment means averaged over five levels of soil moisture and four replications.

soil moisture decreased, Al decreased and Mg increased (Figure 4). While the ponded soils produced the only significantly different level of Al, there was a consistent trend observed. Increasing inability to control moisture status resulted in an early termination of the experiment. A longer time period with adequate control of soil moisture-levels might have produced statistically significant differences in forage Al concentrations at one or more of the other treatment levels also.

The effect of the ponded soil on increasing Al accumulation in the plant is in agreement with the correlation between soil moisture and forage Al observed under grass tetany pasture conditions. The previous greenhouse study indicated no effect of soil moisture on Al, but the soils were not ponded and control of the saturated condition may have been inadequate to produce any effect on Al accumulation.

Potassium, Ca, Na and Zn forage concentrations were all lowered by increasing soil moisture while Mn concentrations were increased. Phosphorous accumulation was highest at the saturated, 100 and 300 cm soil moisture levels and decreased significantly with both ponded and dry soil (600 cm) conditions.

Collection of soil solution samples was problematical and was too incomplete for any statistical analysis. Samples that were obtained were too small for complete chemical analysis. Manganese and Al levels were checked on samples that were available. Samples from ponded soils appeared to contain the highest level of Mn. No soil solution analyzed was found to contain a detectable level of Al.

Root samples were scanned for mycorrhizal infection (Table 15). Roots growing under ponded or saturated conditions contained very few or

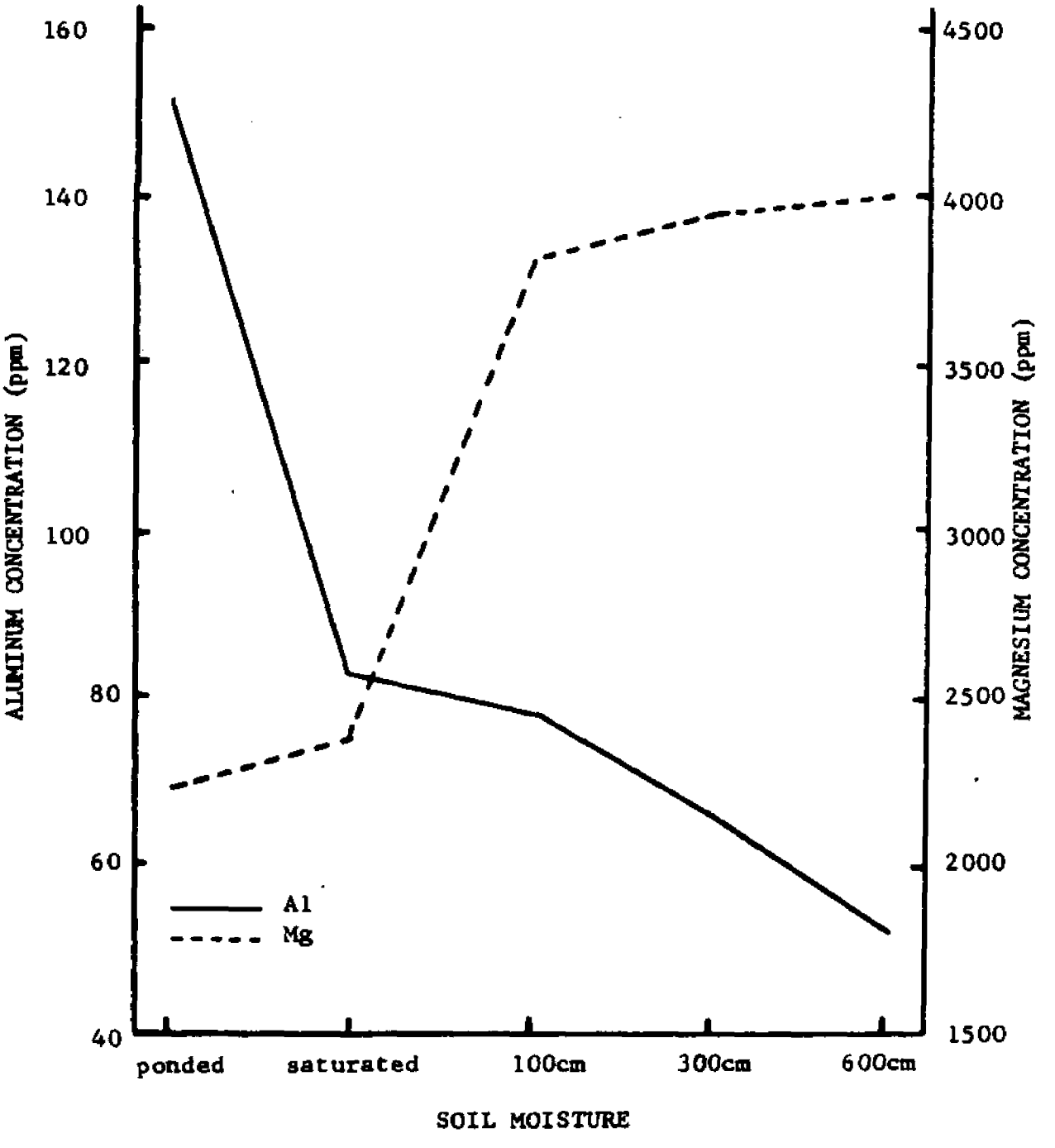

Fig.4. The effect of soil moisture level on the concentration of Al and Mg in ryegrass grown in the greenhouse.

Table 15. Observations of the presence of endomycorrhizal fungi in ryegrass roots under five levels of soil moisture in Falaya silt loam and Mhoon silty clay loam in the greenhouse.

Soil	Treatment	Relative Numbers Present			
		None	Few	Several	Many
Falaya	Ponded	X			
	saturated	X	X		
	100 cm		X	X	
	300 cm			X	X
	600 cm			X	X
Mhoon	Ponded	X			
	saturated	X			
	100 cm	X	X		
	300 cm		X	X	
	600 cm		X	X	

no blue stained fungal hyphae. Roots growing under moisture conditions of 100, 300 and 600 cm suction values contained easily detectable levels of infection. Roots taken from Falaya silt loam appeared to contain a higher degree of infection than those from Mhoon silty clay loam soil. The conditions of this experiment do not allow conclusions to be drawn concerning the relationship of mycorrhizal fungi and the concentration of forage minerals. It can be observed that they were present in higher relative numbers in plants grown in Falaya soil and that these plants did contain higher levels of P and as much Mg as plants grown on the Mhoon soil although the Falaya soil contained a much lower exchangeable Mg level (Table 14). Plants grown on ponded and saturated soils contained very few or no hyphae and lower concentrations of Ca, Mg, and P.

The soil water content was determined in each pot at the end of the experiment to see how effective the control of moisture level had been. The results presented in Table 16 indicate adequate control.

D. Growth Chamber Experiments

1. Temperature and Soil Types

Review of grass tetany literature repeatedly suggest a relationship between temperature and the occurrence of the disease. A drop in temperature below 14 C is associated with a decrease in the number of tetany cases. However, low temperatures followed by temperatures above 14 C have frequently been associated with an increased incidence of grass tetany approximately five days later. Results of a growth chamber experiment designed to study the forage mineral responses to such changes in temperature are presented in Table 17 and Figure 5. Aluminum accumulation was greatly influenced by the exposure to cold temperature

Table 16. Soil water content* in two soil types under five suction values at two depths in greenhouse pots.

Soil Depth (cm)	Treatment	% Moisture in: Falaya silt loam	Mhoon silty clay loam
		g/100g	
0-5	ponded	57.85	56.08
5+		--	--
0-5	saturated	45.13	52.55
5+		--	--
0-5	100 cm	31.22	32.00
5+		27.51	30.24
0-5	300 cm	25.30	26.86
5+		20.72	26.43
0-5	600 cm	15.29	21.82
5+		14.55	20.86

* Average of four replications.

Table 17. The effect of three temperatures on mineral concentrations* of ryegrass grown in a growth chamber.

Temperature	Al	Mn	Zn	Na	Ca	Mg	P	K
	ppm							%
8-12	26a	196a	132a	6681a	1855a	2555a	3756ab	1.4a
7-11	45a	157a	276b	1834b	6771b	2613a	3443a	2.7b
0-3	215b	166a	548c	4641c	7068b	3302b	4207b	3.2b

* Means averaged over five harvest dates and two soils and four replications.

Means within columns followed by the same letter do not differ at the 1% level according to Duncan's New Multiply Range Test.

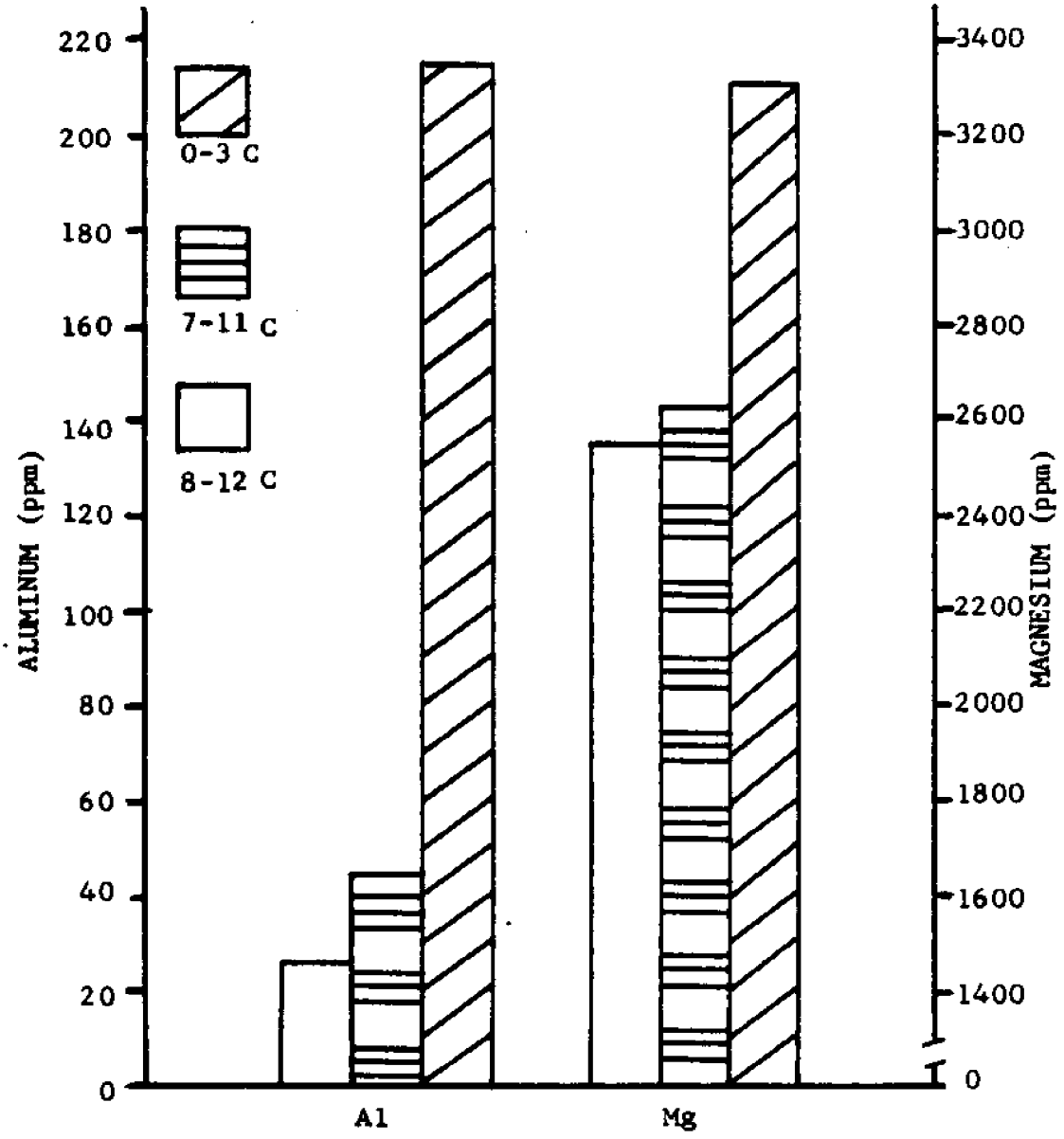

Fig. 5. The effects of cold temperature for 22 days on the accumulation of Al and Mg following a rise in temperature above 14 C in ryegrass grown in a growth chamber.

($p \leq 0.01$). Plants exposed to the coldest conditions (0-3 C) accumulated five to eight times as much Al when temperature was raised above 14 C as those exposed to the 7-11 C, and 8-12 temperature. Magnesium, Zn, Ca, P and K concentrations were also higher in plants exposed to the coldest temperature, but proportional increase was greater for Al. As one would expect due to greater activity of Mn reducing organism at higher temperatures, Mn forage concentrations were higher in plants grown in the warmest conditions ($p \leq 0.05$). Potassium accumulation was lowest in plants exposed to the highest temperature ($p \leq 0.01$).

Table 18 shows the effect of time after cold treatment on mineral accumulation. Two interesting points were observed in the data. Magnesium levels increased, after the temperature rose above 14 C, for a period of about four days. At that point Mg accumulation ceased. No significant differences were found between days 2, 4, 6, and 8. On the other hand, Al steadily increased from day 2 through day 8 (Figure 6).

The five day critical period often referred to in grass tetany literature occurred after Mg levels reached a plateau and while Al levels were increasing at a rapid rate. Manganese, Ca, P, and Na concentrations also increased following the rise in temperature. No effect was found for either Zn or K.

The effects of time of sampling in Table 18 are based on the results obtained from the growth chamber experiment conducted at 8-12 C only. Mechanical problems with the growth chamber during the experiments at the two colder temperatures, make the data obtained during this trial most reliable.

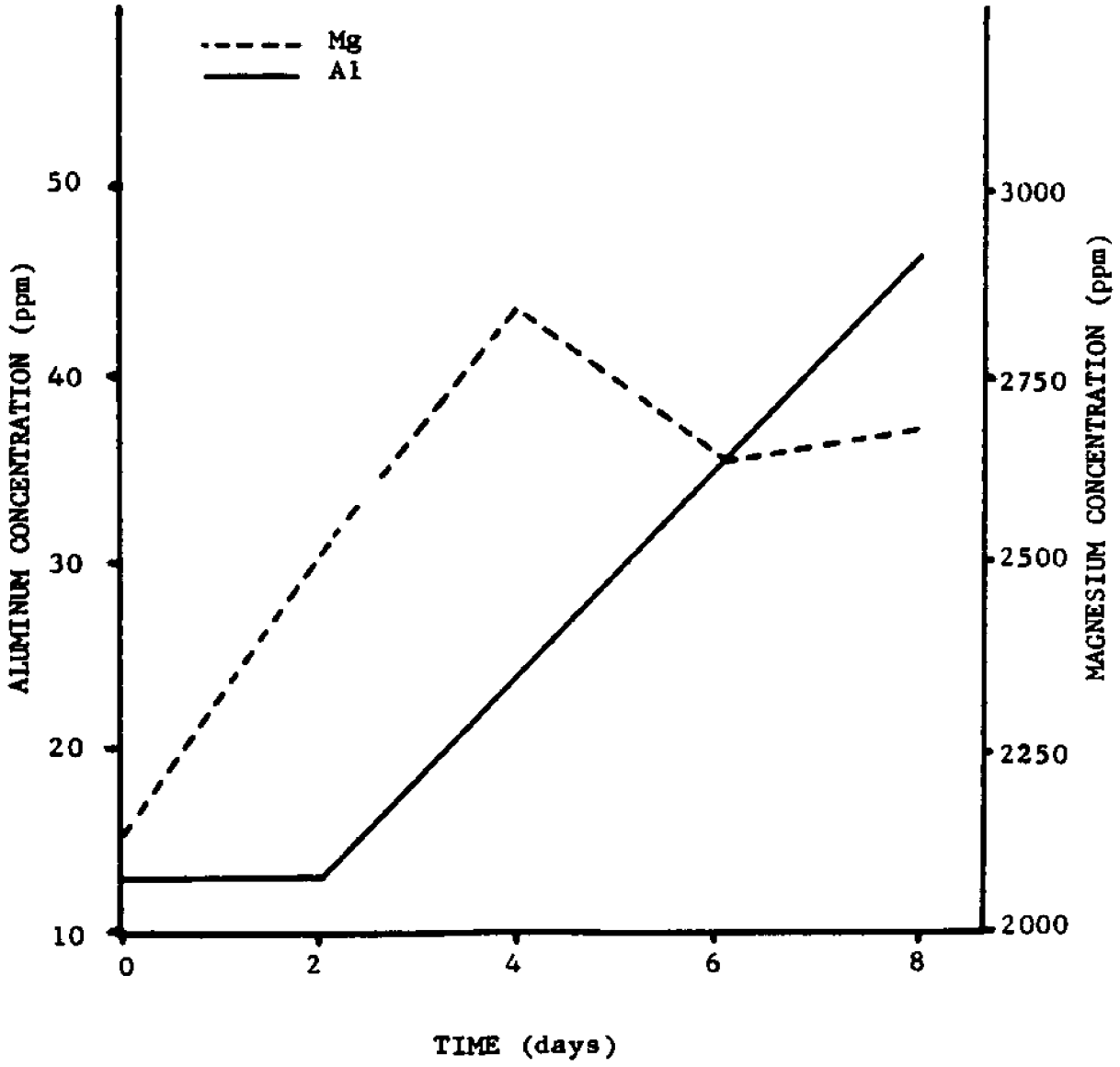

Fig. 6. The concentration of Al and Mg in ryegrass as a function of time following a rise in temperature to above 14 C in the growth chamber.

Table 18. Mineral concentrations* in ryegrass grown in a growth chamber at 8-12 C as a function of time following a rise in temperature to above 14 C.

Days after cold treatment	Al	Mn	Zn	Na	Ca	Mg	P	K
	ppm							%
0	13a+	126a	123a	5651a	1125a	2112a	2375a	1.2a
2	13a	163ab	139a	6467b	1637ab	2493ab	3381b	1.4a
4	24b	210cb	148a	6843bc	2231c	2843b	4350c	1.4a
6	35b	228c	120a	7043dc	2156cb	2643b	4200c	1.6a
8	46c	253c	131a	7398d	2125cb	2681b	4475c	1.4a

* Means averaged over two soil types and four replications.

+ Means followed by the same letter within columns do not differ significantly at the 1% level according to Duncan's New Multiple Range Test.

2. Temperature, Soil Type and Moisture Level

Another growth chamber experiment designed to study the effect of two soil moisture levels on mineral concentrations in ryegrass could not be completed under the intended conditions. The only result obtained from this experiment was to substantiate the effect of ponded soils on increasing Al and Mn accumulation and decreasing K and Mg concentrations in ryegrass forage (Table 19).

Soil solution samples from the ponded soils were analyzed for Al. Traces of Al were detectable (< 0.5 ppm) in soil solutions collected on the first harvest date while still exposed to cold temperature.

Table 19. Effect of two soil moisture levels on mineral accumulations in ryegrass* under growth chamber conditions.

Soil Moist Level	Al	Mn	Ca	Mg	P	K
	ppm					%
Ponded	203a+	157a	8686a	2440a	3626a	2.4a
100cm	137b	205b	9251a	2976b	3426a	4.2b

* Means averaged over two soils and five sampling dates and four replications.

+ Means within columns followed by the same letter do not differ significantly at the 5% level according to Duncan's New Multiple Range Test.

No difference could be found between Falaya and Mhoon soil solutions. No detectable levels of Al were found in soil solutions collected from either soil at any time after the temperature was raised above 14 C.

E. Bickham Pasture Investigation

Of the four field sites (A, B, C, and D) in the Bickham pasture, site D maintained the highest level of soil moisture throughout the five-month sampling period. The forage samples from that plot also contained the highest level of Al. Aluminum concentration peaked at 1090 ppm on February 21. As shown in Figure 7 this peak occurred about a month after ponded conditions developed. The coldest soil temperature (3 C) was recorded on February 1. Three days later forage Al

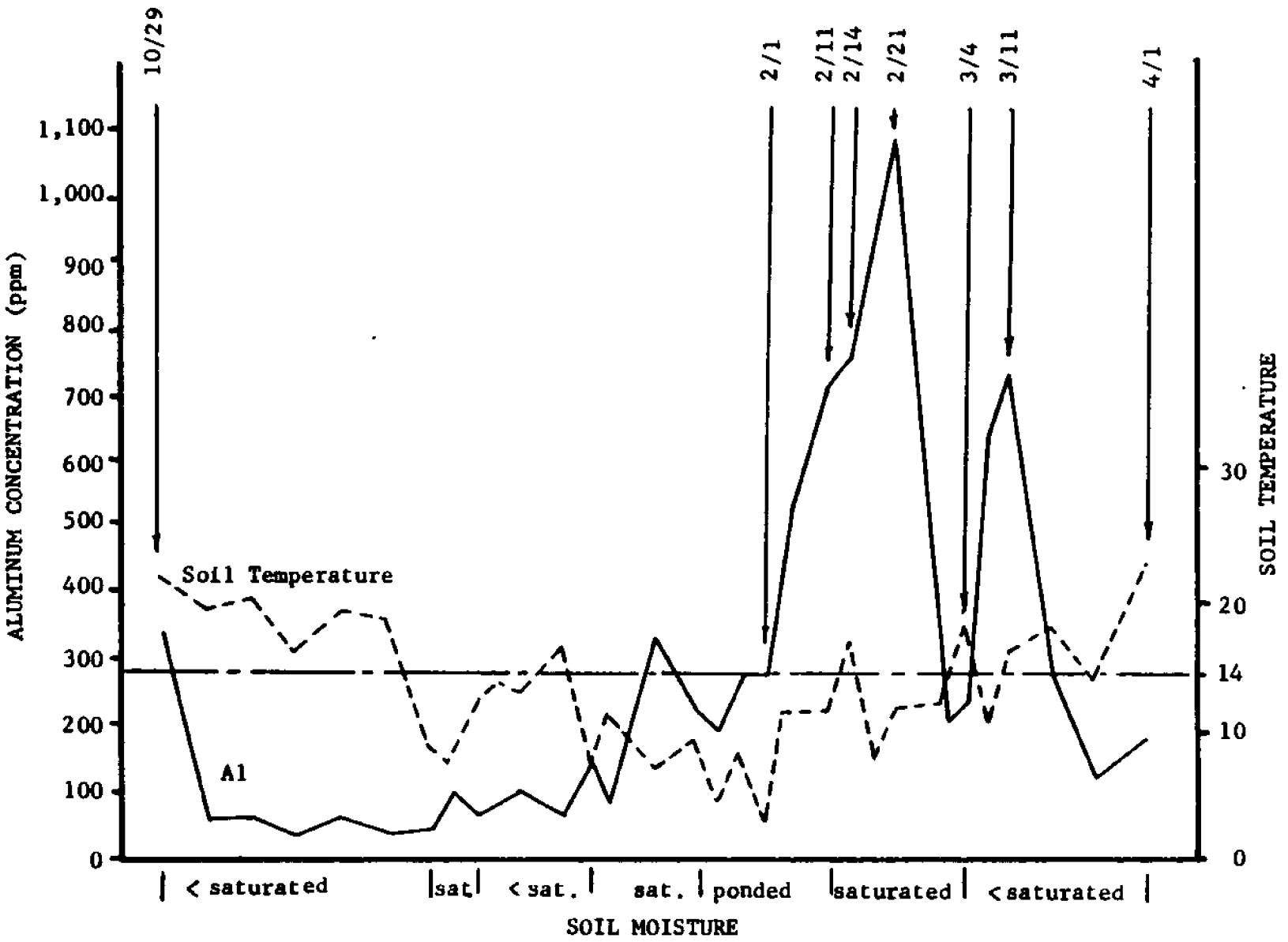

Fig. 7. Forage Al concentration, soil temperature and visible moisture status at site D during five month sampling period in the Bickham Pasture.

concentrations had significantly increased. By February 14 the recorded soil temperature had risen to 16 C (above the 14 C critical point in grass tetany research) and seven days later forage Al concentration reached its highest level of 1090 ppm. Site D was under ponded conditions with a thin ice cover on February 1 while February 14 was very warm, spring-like weather. Grass had grown visibly since the last sampling date (February 11). These conditions of a ponded soil and cold temperatures followed by warm temperature and a rise in forage Al concentration are in agreement with the results obtained in the growth chamber and greenhouse experiments.

A second smaller Al peak occurred on March 11 (720 ppm Al). This peak was preceded by a low temperature of 8 C on February 18 and a subsequent rise in temperature to 18 C on March 4. Again the Al peak occurred seven days later. The peak was lower and the coldest soil temperature was higher than in February but still below the 14 C critical point. A saturated soil moisture condition existed at the second peak of Al accumulation. By the time the soil temperature rose above 14 C for the last time on March 11, the soil moisture level was below saturation. The preceding low was 11 C. The temperature effect observed at this location appears similar to the effect observed in the growth chamber in that a temperature of 0-3 C resulted in higher Al accumulation than a low temperature of 8-12 C.

Site D was continually under ponded conditions from January 21 through March 1, 1979. On March 8 it was below saturation levels for the first time since December 31, 1978. On March 11 this location was again near saturation conditions but by March 18 the area

was well below saturation. Saturation was not approached again throughout the remainder of the experiment. When the soil moisture dropped below the saturation level and soil temperature stayed above 14 C, forage Al concentration dropped and remained below 200 ppm.

None of the other three locations were under ponded conditions at any time. Saturated soil conditions were noted but were of very short duration. Laboratory determinations of soil moisture indicated that location B maintained slightly higher soil moisture levels than the other two locations and that location A maintained the lowest levels of soil moisture. All three of these locations showed increased Al concentration during the coldest period and all three locations reached their highest recorded level of Al on the same day, February 11. This was ten days after the coldest recorded soil temperature. The Al concentrations recorded at locations A, B and C on this day were 570, 320 and 44 ppm, respectively. The Al level at location D increased to 710 ppm on this day but continued to rise for ten more days.

The correlation coefficients between forage Al accumulations and other mineral concentrations are shown in Table 20. Manganese and Al concentrations in forages had the highest and most consistent correlations among the four locations. It is entirely possible, however, that this is due to both Al and Mn being highly correlated to some other factor. A significant negative correlation was found at locations B and D between forage Mg and Al concentrations. The correlation with Ca was also negative and was significant at three locations. Phosphorus had a significant positive correlation with Al at three of the four locations.

Table 20. Correlation coefficients between forage Al accumulations and the recorded variables at four locations in the Bickham research field.

Variable	Location A	B	C	D
Soil temperature	-0.05	-0.24	-0.17	-0.15
Soil moisture	0.28	0.45**	0.25	0.44*
Air tempertures				
7 day high ave.[1]	-0.20	-0.37*	-0.43*	-0.39*
7 day low ave.[2]	-0.13	-0.25	-0.30	-0.32
7 day low[3]	-0.03	-0.17	-0.17	-0.19
mean[4]	-0.17	-0.33	-0.39*	-0.37*
5 day mean[5]	-0.19	-0.26	-0.34	-0.33
5 day low[6]	-0.06	-0.14	-0.23	-0.25
2 day low[7]	-0.27	-0.34	-0.36	-0.35
1 day low[8]	-0.03	-0.24	-0.24	-0.23
Forage Mineral Concentration				
Mn	0.93***	0.67***	0.63***	0.71***
Mg	-0.12	-0.39*	0.17	-0.46**
Ca	-0.29	-0.52**	-0.44*	-0.53**
P	0.44*	0.27	0.59***	0.51**
K	-0.16	-0.07	-0.29	0.08

*, **, ***, denotes significance at the 5, 1, and 0.1 % level.

[1]Average of daily high temperatures for seven days prior to sampling.

[2]Average of daily low temperatures for seven days prior to sampling.

[3]Lowest single temperature which occurred during the seven days prior to sampling.

[4]Mean of the daily high and low temperatures of the seven days prior to sampling.

[5]Mean of high and low temperature which occurred on the fifth day prior to sampling.

[6]The lowest temperature which occurred on the fifth day prior to sampling.

[7]The lowest temperature which occurred on the second day prior to sampling.

[8]The lowest temperature which occurred one day prior to sampling.

Soil moisture, recorded in g/100g (Table 20) was significantly correlated with Al accumulation at the two wettest locations (D and B). Soil moisture measurements, made by drying soil samples, probably did not reflect sufficient differences between saturated and ponded conditions to produce the highest possible correlations. A significant difference in the effect of saturated and ponded soils on Al concentration was found in the greenhouse experiment. The average of the daily high air temperatures for the seven days prior to sampling showed the most significant air temperature correlation with Al accumulation. The relationship was negative at all locations and significant at three of the four locations. The second best air temperature correlation with Al accumulation was the mean which was calculated as the mean of the daily high and low readings averaged over the seven day period preceding sampling. A significant negative relationship between this mean air temperature and Al accumulation was found at locations C and D. This relationship at location B approached significance. While not statistically significant, the lowest air temperature that occurred 24-48 hours before sampling had a stronger correlation with Al accumulation than either the lowest temperature within 24 hours prior to sampling or the lowest temperature five days prior to sampling. Although not included in this study, it is possible that a stronger correlation exists between Al accumulation and the maximum air temperature 24-48 hours prior to sampling. A low maximum temperature may have more influence on Al accumulation than the minimum temperature. Soil temperatures, particularly those occurring two to seven days prior to sampling may be more closely associated with Al accumulation than air temperatures, but

the lack of an instrument to continuously monitor soil temperature prevented collection of this data except at the time of sampling.

The correlation coefficients between Mn concentration and with the measured independent variables are given in Table 21. Again, no particularly high correlations were seen except the previously mentioned correlation with Al. Magnesium, significantly correlated with Mn at sites C and D, showed a non-significant negative relationship at the other two sites. The correlation on Mn with soil moisture was significant at location D.

The data obtained in this research were subjected to a stepwise multiple regression procedure utilizing a maximum R square improvement technique in an attempt to identify possible combinations of variables which were influencing Al accumulation. The multiple regression was run first to include environmental effects and again to include both environmental and mineral concentration effects. Regression coefficients are given in Tables 22 and 23. When the analysis was performed with the variables of environmental conditions, soil temperature was implicated at three of the four locations. Correlations were relatively low, however. When all of the variables were entered in the analysis, the correlations were much higher but little or no consistency was found between locations. More meaningful results could probably be obtained from this type of analysis with a more controlled experiment and the inclusion of other unmeasured variables, such as continuously monitored soil temperatures.

F. In Vitro Experiments

Addition of Al to the in vitro digestion systems was very

Table 21. Correlation coefficients between forage Mn accumulations and the recorded variables at four locations in the Bickham research field.

Variable	Location A	B	C	D
Soil temperature	-0.11	0.03	0.13	0.04
Soil moisture	0.29	0.35	0.25	0.47**
Air temperatures				
7 day high ave.[1]	-0.25	-0.17	-0.08	-0.22
7 day low ave.[2]	-0.12	0.08	0.06	-0.03
7 day low[3]	-0.07	0.12	0.15	0.05
mean[4]	-0.20	-0.06	-0.02	-0.14
5 day mean[5]	-0.22	-0.03	-0.04	-0.14
5 day low[6]	-0.09	-0.02	-0.02	-0.06
2 day low[7]	-0.28	-0.09	-0.17	-0.24
1 day low[8]	-0.06	-0.07	-0.05	-0.06
Forage Mineral concentration				
Al	0.93***	0.67***	0.63***	0.71***
Mg	-0.18	-0.05	0.59***	-0.57***
Ca	-0.38*	-0.02	-0.35	-0.40*
P	0.44*	0.24	0.62***	0.47***
K	-0.16	-0.02	-0.06	0.17

*, **, *** denotes significance at the 5, 1, and 0.1% level.

[1]Average of daily high temperatures for seven days prior to sampling.

[2]Average of daily low temperatures for seven days prior to sampling.

[3]Lowest single temperature which occurred during the seven days prior to sampling.

[4]Mean of the daily high and low temperatures of the seven days prior to sampling.

[5]Mean of high and low temperature which occurred on the fifth day prior to sampling.

[6]The lowest temperature which occurred on the fifth day prior to sampling.

[7]The lowest temperature which occurred on the second day prior to sampling.

[8]The lowest temperature which occurred one day prior to sampling.

Table 22. The best variable model found in the stepwise regression of Al with temperature and soil moisture variables in the Bickham pasture.

Location	Variables entered	R Square	r	Prob> F
A	Soil temperature	0.2337	0.4835	0.070
	Soil moisture			
	2 day low air temperature			
B	Soil moisture	0.2046	0.4523*	0.012
C	Soil temperature			
	Air temperature high (7 day ave)	0.3107	0.5574**	0.009
D	Soil temperature			
	Air temperature high (7 day ave)	0.2508	0.5008*	0.020

*, **, ***, denote significance at the 5, 1, and 0.1% level respectively.

Table 23. The best variable model found in the stepwise regression of Al with all variables of temperature, soil moisture, and forage mineral concentrations measured in the Bickham pasture.

Location	Variables entered	R Square	r	Prob> F
A	Soil temperature	0.92	0.96***	0.001
	Mn			
	Mg			
	P			
	K			
B	Mn	0.77	0.88***	0.001
	Ca			
	7 day low average (air temp)			
C	P			
	K			
	Soil moisture	0.75	0.87***	0.001
D	Mn			
	7 day low average (air temp)	0.62	0.79***	0.001

*, **, *** denote significance at the 5, 1, and 0.1% level respectively.

effective in removing Mg from solution as shown in Table 24. Mean differences due to Al additions were highly significant ($P \leq 0.01$) in each of the three combinations of ryegrass, rumen fluid, and buffer solution, differing both from the control and the two Mn treatments. Addition of the high and low levels of Al to the ryegrass-rumen fluid-buffer solution combination removed 47 and 56% of the Mg from solution, respectively. The two levels of added Al gave results that were significantly different from each other except when added to the buffer solution only. Addition of Al plus Mn was no more effective in reducing Mg solubility than the addition of Al alone.

The addition of Al to the in vitro systems had even greater effects on Ca solubility than it had on Mg solubility. The low and high levels of added Al in the ryegrass-rumen fluid-buffer solution combination removed 64 and 74% of the Ca from solution, respectively. Both levels of added Al significantly reduced Ca solubility in each combination of ryegrass, rumen fluid, and buffer solution. Effects of levels of Al differed from each other only in the first combination. In the second and third combinations, nearly all Ca was removed from solution by each level of added Al.

The addition of Mn to the in vitro systems significantly reduced Mg solubility only when added to tubes containing ryegrass, rumen fluid, and buffer solution. In that system, each level of added Mn significantly reduced Mg solubility. The high level of added Mn reduced Mg solubility (40%) more than the low level of Mn (37%), but less than the low level of added Al (47%). Additions of Mn reduced Ca solubility in each of the three in vitro systems tested. However, the two levels of

Table 24. The influence of Al and Mn on the solubility of Mg and Ca in combinations of ryegrass, rumen fluid and buffer solution during in-vitro incubation.

Treatment	Ryegrass, rumen fluid and buffer		Rumen fluid and buffer		Buffer	
	Mg	Ca	Mg	Ca	Mg	Ca
	ppm					
control	35.50a*	20.70a	8.85a	7.85a	2.95	1.55a
Mn (1)	22.20b	11.95b	8.25a	6.30b	2.80a	0.45b
Mn (2)	21.45c	10.95c	8.35a	6.25b	2.95a	0.55b
Al (1)	18.90d	7.45d	5.45b	3.10c	0.90b	0.00b
Mn + Al (1)	18.70d	7.05d	5.85b	3.45c	0.75b	0.00b
Al (2)	15.65e	5.35c	4.25c	2.75c	0.60b	0.00b
Mn + Al (2)	15.80e	4.80c	4.40c	2.35c	0.60b	0.00b

* Means within columns followed by the same subscript do not differ significantly at the 1% level according to Duncan's New Multiple Range Test.

Mn differed significantly in their reduction of Ca solubility (42 and 47%), only when added to the system containing ryegrass, rumen fluid, and buffer solution ($P \leq 0.05$). Manganese treatment effects on Ca solubility differed from Al treatment effects at the 1% level in the first two systems and at the 5% level in the third system tested.

G. In Vivo Experiments

1. Fistulated Steers

The effect of added Al and Mn to the rumen of four fistulated

steers is shown in Figure 8. Serum Mg levels declined within 24 hours after Al treatments began and continued to fall at the same rate over the four-day treatment period. The response to both Al and Al plus Mn was rapid and continuous. There was no indication of a leveling off of response at the time the treatments were terminated. Treatments were terminated in order to allow the animals to recover so that the Latin Square experiment could be repeated four times. Within twenty-four hours after treatments were discontinued serum Mg levels began a rapid return to normal. The effects of Al treatment were the same with or without the addition of Mn. Although not shown in Figure 8, the serum Mg levels for the Al- and Al plus Mn-treated steers at the end of the ninth day were 17.44 and 17.71 ppm, respectively. At that point blood levels were no longer significantly different from the controls.

A drop in serum Mg under normal conditions is counterbalanced by a concomitant rise in serum Ca. A continued fall in serum Mg appears to affect Ca balance however, and result in a fall in serum Ca levels also. Clinical symptoms of grass tetany are often noted to occur at that point. The treatment period of this experiment was probably not long enough to interrupt Ca balance. Visible clinical symptoms of hypomagnesemics in these animals were limited to loss of appetite and grinding of the teeth. Slight shivering was noted occasionally but this may have been due to the cold weather and low feed intake.

Rumen fluid Al levels were found under normal conditions to be less than 1 ppm or undetectable in this experiment. Introducing Al into the rumen dramatically increased the amount of soluable Al (Figure 9). The return of Al to normal levels in rumen fluid roughly corresponded

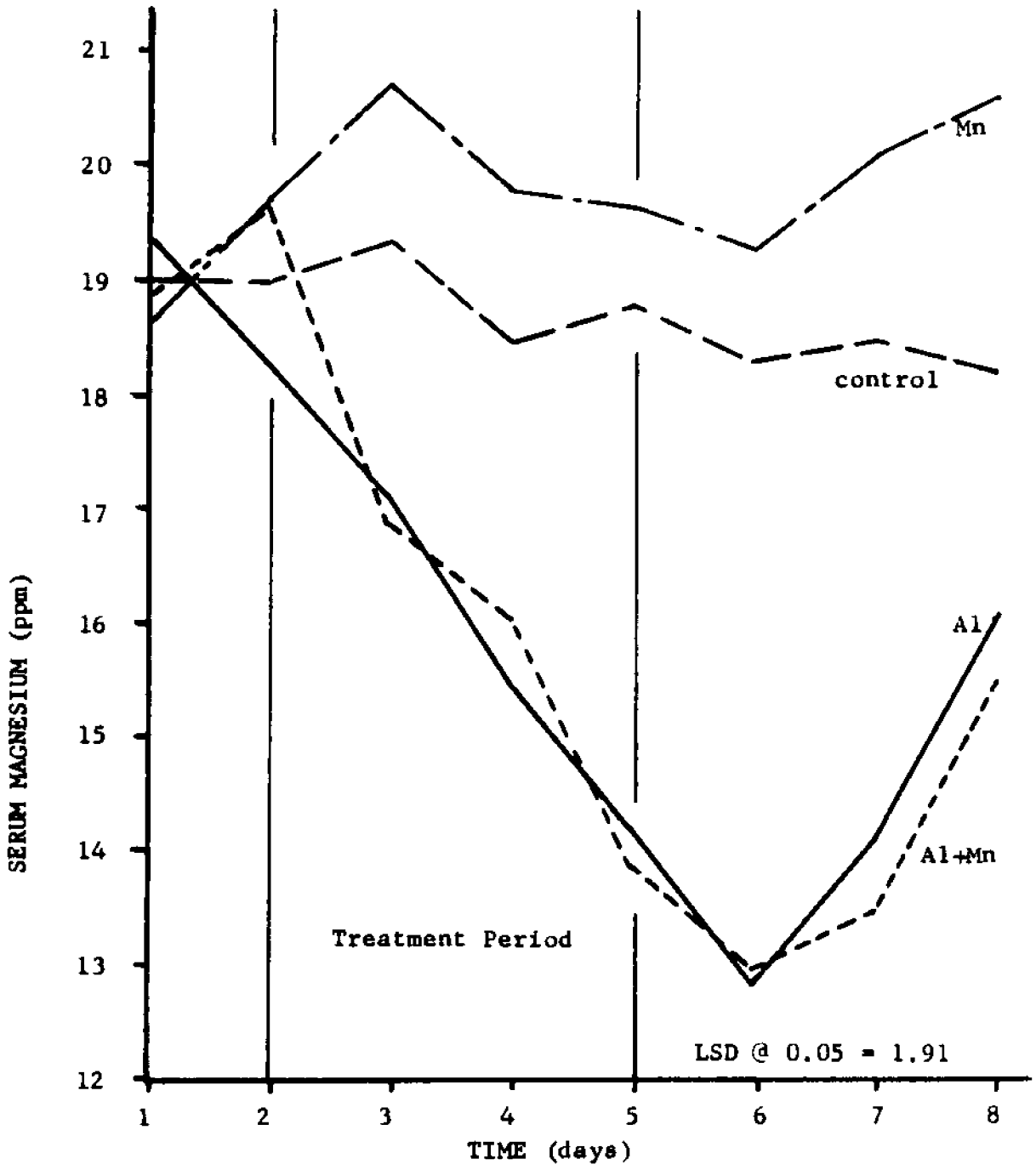

Fig. 8. The effect of adding Al and Mn to the rumen on average serum Mg levels in fistulated steers.

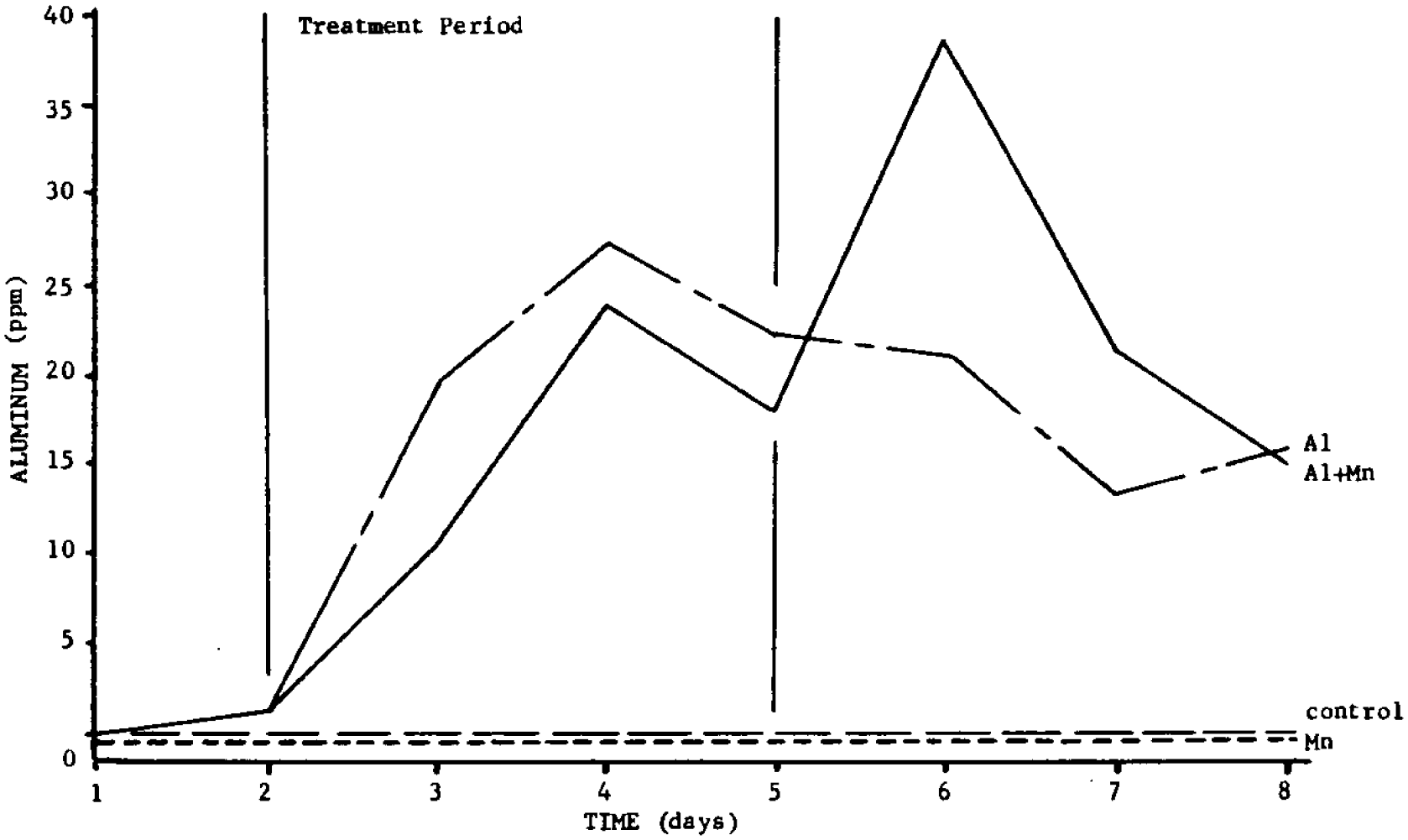

Fig. 9. The effect of added Al on the concentration of soluble Al in the rumen fluid of fistulated steers.

to the return of serum Mg to a normal level. The rumen fluid concentration of Mg, Ca, Al, and Mn was highly variable due to the effects of variation in animal intake of feed and water. No attempt was made to adjust the values to compensate for this source of variation.

Rumen fluid pH values were found to range from 6.7-7.0 in control animals. Mn had no apparent effect on rumen fluid pH. Aluminum treatment generally tended to raise pH values to a range of 7.1 to 8.1. However, pH values dropped as low as 4.5 in one animal treated with both Al and Mn during one replication of this experiment. This particular animal ceased to consume both feed and water.

2. Lactating Cows

The results obtained from administering Al and Mn to lactating cows is presented in Figure 10. Serum Mg levels in control animals generally increased during the experimental period. Twenty-four hours after treatment began, serum Mg in the control animals were significantly higher than in either group treated with Al, and remained higher throughout the entire test period L.S.D.(0.01). The group receiving Al and Mn exhibited significantly lower serum Mg on the last day of treatment (day 5) and again on the last day of the experiment (day 8) than did the group receiving Al only. The immediate fall and recovery of serum Mg in response to administered Al and Mn was not observed in the cows, however, as it was in fistulated steers. The lack of immediate response may be due to differences in the technique of dosing. Placement of the Al into the ventral sac via rumen cannular directly into the rumen fluids may have initiated a quicker response than drenching via stomach tube. The lactating cows may have also responded

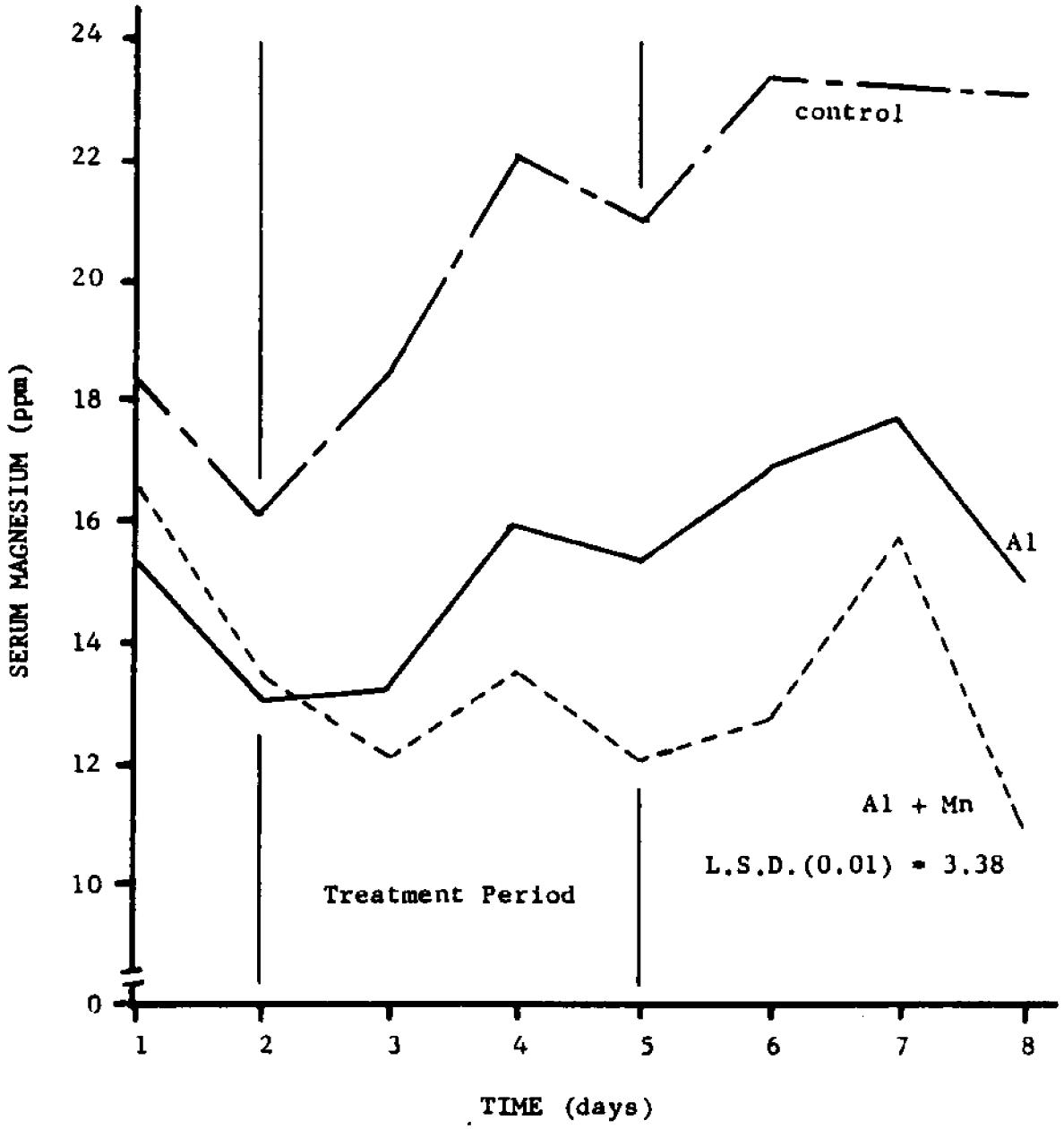

Fig. 10. The average effect of orally administered aluminum and manganese on serum Mg levels of lactating cows.

differently due to the different physiological status and to the lack of restriction on dietary intake.

Both Al-treated groups showed a sharp drop in serum Mg during the last 24 hours of the test period. Whether or not this was the beginning of a delayed effect of Al levels within the rumen is not known since no rumen fluid samples were obtained. Problems encountered with serum analysis technique also undoubtedly effected the results obtained from this experiment. Analysis of these samples was begun using a 1:11 serum dilution with distilled water. This technique produced unstable readings by atomic absorption spectroscophy. Beginning on day 4 samples were frozen until a method was developed using 5% HCl in distilled water for dilution. Samples for the remainder of the experiment were then thawed and analyzed by the new technique. This method resulted in stable but slightly higher analysis values (Appendix Table 27). Two samples were lost due to breakage in the freezing process.

DISCUSSION

Grass tetany occurred in different geographical locations including two states. Pastures were on a number of different soils and were composed of different species of forage plants. Tetany occurred in animals fed hay as well as pasture and in both pre- and post-parturient cattle that represented several breeds and crossbreeds. The only factor common to all cases was high Al accumulations in the rumen contents. Areas of high forage Al accumulation were frequently associated with high soil moisture levels, an effect further documented in greenhouse experiments. Growth chamber studies showed that temperature alone affected forage Al concentrations, but the effect was more pronounced on ponded than on aerated soils. The four sites in the Bickham pasture were subjected to essentially the same temperature conditions but Al accumulation was two or more times higher under ponded conditions than non-ponded conditions. It was concluded that Al accumulation was influenced by both moisture and temperature but the effect was greater under the combined influence of both factors.

Soil pH appeared to have no relationship to forage Al accumulations. Plants accumulated high Al levels on both acid and alkaline soils. This is in contrast to generally accepted concepts concerning pH and Eh or oxidation-reduction effects on Al activity.

The situation which allows high levels of Al to enter the plant may be a soil effect which brings large quantities of Al into an "available" state. It may also be a plant effect which allows the normally excluded Al to enter the plant in large quantities. The

possibility also exists that it is the result of influences which cause either the proliferation or exclusion of certain microorganism, thereby allowing the uptake of Al by the plant.

The pH measured in the soil samples may bear little resemblance to the pH of the root surface sorption zone. The pH in this minute area, affected by mocroorganisms and the plant root itself, may well be the critical pH in Al accumulation (45). It is doubtful if this alone is the explanation although it may be an important part of the total picture. The reducing conditions and cold temperatures under which this phenomena occurs may also effect plant uptake mechanisms. Respiration is apparently essential for maintaining differential membrane permeability and the rate of respiration can be reduced by both low oxygen and low temperature. This effect may account for the difference in Al uptake between ponded and saturated moisture conditions. Under saturated conditions there could still be pockets of aeration while ponded conditions should exclude more total oxygen. This effect on membrane permeability could also explain why Al concentrations increased in response to temperature under both reducing and oxidizing conditions.

Undoubtedly, one aspect of grass tetany is reduced Mg uptake which involves microorganisms. The depression of Mg accumulation by plants under conditions of low soil oxygen may be due in part to the exclusion of specific microbes. Endomycorrhizal fungi, which are thought to increase Mg uptake, are excluded under reducing conditions. Nitrogen in the nitrate form undergoes microbial denitrification. Ammonium ions are known to depress Mg uptake while NO_3 as well as PO_4 and SO_4 ions are

known to enhance it. The SO_4 compounds would also undergo microbial reduction under high moisture conditions. Reducing conditions favor microbial conversion of Mn^{+4} to the more soluble Mn^{+2} form. This conversion may further reduce Mg uptake by plants in the manner described by Maas, et al. (56). The role microorganisms play in Al accumulation is yet to be seen.

High levels of Al are often expressed as a phosphate deficiency due to a depression of P absorption and translocation within the plant. However, symptoms of PO_4 deficiency were not found in tetany pastures, even in the area where plants contained over 14,000 ppm Al. This result indicates the Al was not predominately in the PO_4 form. One mechanism that some Al-tolerant species employ is an organic acid buffer system. Aluminum in the form of several organic acids has been shown to exist in certain plants. High levels of citric and trans-aconitate have been studies in relation to grass tetany. The form in which Al is ingested by the animal may be important to its effect on the animal.

Aluminum ingested by ruminants could effect Mg balance in at least four possible ways. The first, as shown in the in vitro study, by reducing Mg solubility, thereby rendering it unavailable for absorption. Ruminants are highly dependent upon absorption for maintenance of Mg balance. The second possibility is that Al is toxic to rumen microorganisms and is a deterent to microbial decomposition of ingested feed and thus limits the amount of Mg brought into solution. A third possibility exists in an interference with the absorptive mechanism or mechanisms for Mg within the rumen mucosa. Whatever the mechanisms

involved, Mg absorption is not thought to be attributable to passive absorption. The fourth possibility is that at high levels of Al intake, absorption of Al itself may occur. Absorbed excessive Al has been shown to interfere with glycolysis. In fact, ketosis, which results from the accumulation of two carbon fragments from fat catabolism due to a breakdown in glycolysis, is often noted to accompany cases of grass tetany. It may be that the immediate effect of excess ingested Al is through percipitation of Mg in the rumen, resulting in a rapid decline of serum Mg levels. A response was seen within 24 hours after Al was administered in the fistulated steers. The onset of clinical tetany symptoms, however, may wait until the Ca balance is also affected. Magnesium apparently facilitates the release of Ca from bones in the presence of adequate amounts of vitamin D and PTH. An absolute lack of Mg, then, could bring about a hypocalcemic condition. It is also possible that the impaired use of the Mg that is present could have the same results. It is interesting to note that biological reactions involving ATP and Mg are thought to be targets of excess Al and that one of the principal sites of deposition of excess Al is in bone. If Ca mobilization mechanisms become impaired, then a much greater importance is placed upon Ca absorption for maintenance of balance. Calcium solubility was affected by the presence of Al even more than Mg in the <u>in vitro</u> work.

Manganese could become a contributing factor to this system of balances through its microbial effect on the depression of VFA's and the reduction of cellulose decomposition, which further complicates the energy situation. Manganese was also shown <u>in vitro</u> to have some ability to remove Ca and perhaps Mg from solution. There is also a possibility

for direct competition for absorption sites between Mn and Mg within the rumen mucosa.

SUMMARY AND CONCLUSIONS

An investigation was conducted in pastures grazed by animals diagnosed to have grass tetany. Eleven locations were included in the study, ten in Louisiana and one in Tennessee. Chemical analysis of the forage indicated that pasture forages were generally low in Mg (0.20%), contained less than 3% K, and were high in Al concentrations. Aluminum values commonly ranged from 2,000 to 8,000 ppm. One location was found to exceed 14,500 ppm Al. The accumulation of Al did not occur uniformly within pastures; instead a wide range of concentrations were found among sampling sites within pastures. The measured soil pH value at the sites of highest Al accumulation ranged from 5.1 to 7.3 and did not appear to be a related factor. Exchangeable Al in the soil surface horizons was not detected. Highest forage Al accumulations were observed in the wettest areas of the pastures. Chemical analysis of the rumen contents of animals that died from grass tetany revealed concentrations of Al more than five times the concentrations found in normal fistulated cattle grazing non-tetany pastures.

An investigation was conducted to determine the effect of P and different sources of Mg on the concentration of Mg in forage dry matter. The results of this experiment were limited by a prolonged fall drought, delayed pasture established, and delayed fertilizer application. Under the conditions of this experiment P did not increase the Mg concentration in forages. A significant increase in Mg concentration was achieved by $MgSO_4$ application at two of the three locations.

A greenhouse investigation was conducted to determine the effects

of two soil moisture conditions (optimum and saturated) and Endogone sporocarps mycorrhizal fungi on the mineral concentrations in ryegrass forage. Plants inoculated with fungi did not differ from controls in mineral concentration. Infection of controls by native fungi strains was as extensive as the level of infection in the inoculated group. Plants grown on saturated soils contained no visible fungal hyphae in their roots and were significantly lower in Mg and Ca and higher in Mn concentration than controls. It is suggested that the absence of fungi might be a contributing factor to the lower observed concentrations of Mg and Ca in these plants. No differences were detected in Al concentration among these three groups of plants.

A second greenhouse investigation was conducted to further investigate the effect of five soil moisture conditions on mineral concentrations in ryegrass forage. Plants grown on ponded soils contained higher concentrations of Al in the forage dry matter than plants grown at any of the drier soil conditions. Forages grown on ponded and saturated soils contained more Mn and less Mg than those grown on drier soils. No differences were found between the two soils in their effects on forage concentrations of Al and Mg.

Growth chamber studies were conducted to investigate the effects of cold temperature exposure on mineral concentrations in ryegrass following a rise in temperature above 14 C. This effect was investigated under two levels of soil moisture (ponded and 100 cm suction) and on two soil types. The coldest temperature was found to result in the highest accumulation of Al during the eight days following a rise in temperature. Aluminum concentrations were higher in forages grown on ponded soils

than in those grown at 100 cm of suction. In the growth chamber experiment that was least influenced by mechanical problems, a linear increase in Al concentration was observed from day two through day eight of the sampling period. Magnesium concentration peaked on day four of the sampling period. No significant difference was found between the two soils in their effect on Al and Mg forage concentrations.

Forage mineral concentrations, soil moisture, soil temperature and air temperatures were monitored throughout the tetany season at four sites within a tetany-prone pasture. Aluminum concentrations exceeded 1,000 ppm at only one site. This high concentration occurred seven days after the soil temperature rose from 3 C to above 14 C and while the soil was under a ponded moisture condition. The other three locations did not develop ponded conditions and were rarely saturated. The highest concentrations of Al found in forage at any of the other three locations was 570 ppm.

An in vitro experiment was conducted to determine the effects of added Al and Mn on the solubility of Mg and Ca in various combinations of ryegrass, rumen fluid and buffer solution. Aluminum reduced Mg in solution by 56% and Ca by 74% in the combination of ryegrass, rumen fluid and buffer solution. Manganese reduced Ca in solution when added alone but had no effect on Mg or Ca when added with Al.

An in vivo experiment was designed to investigate the effect of ingested Al and Mn on blood serum Mg and Ca in fistulated steers. Serum Mg levels in Al-treated steers dropped within 24 hours after treatment began and declined 32% by the end of four days. After treatments were discontinued serum Mg levels returned to normal. Manganese

had no significant effect on serum Mg level.

A second *in vivo* investigation was conducted to determine the effects of ingested Al and Mn on serum Mg and Ca in lactating cows. Serum Mg levels in Al-treated cows were significantly lower than controls 24 hours after treatment began and remained lower than the control group until the experiment was terminated after eight days.

Conclusions

Unexpectedly high levels of Al were found to occur in pasture forages and rumen contents where grass tetany was diagnosed. The areas of highest Al accumulation appeared to occur under conditions of high soil moisture. Forage Al concentrations were increased experimentally in response to low temperatures and high soil moistures. Aluminum drastically reduced the solubility of Mg and Ca in rumen fluid, ryegrass and buffer solution combinations *in vitro*. Aluminum introduced into the rumen of fistulated steers and lactating cows significantly lowered serum Mg levels within 24 hours. Based on field, greenhouse, growth chamber, *in vitro* and animal responses, it appears that Al is actively involved in the etiology of grass tetany. If, indeed, Mn is a part of the pathology, its role is more likely to be that of a contributory factor.

APPENDIX

Table 25. The chemical analysis of forage samples collected in pastures grazed by animals with a diagnosis of grass tetany.

Pasture	Site	Al	Mn	Zn	Na	Ca	Mg	P	K	Soil Moisture
		ppm								%
1	1	3050	270	33	820	4150	1500	2850	2.8	ponded
	2	4140	240	39	810	4650	1800	3100	2.6	ponded
	3	3430	220	34	900	4500	1600	2750	3.3	ponded
2	1	980	380	26	640	2300	1100	2700	2.4	saturated
	2	1310	200	30	560	900	950	2800	2.4	saturated
	3	2200	1840	34	990	1400	1050	3100	2.6	ponded
3	1	720	170	16	280	2850	1050	1900	3.0	saturated
	2	1730	120	22	300	3550	1100	2050	2.8	wet
	3	970	120	17	280	3350	1200	2200	3.4	saturated
4	1	1240	100	24	590	2600	1350	2600	3.1	saturated
	2	1280	110	27	610	2300	1450	2950	2.9	ponded
	3	1620	120	26	350	2450	1450	2700	3.4	saturated
	4	840	110	25	190	2850	1300	2900	2.6	wet
5	1	410	100	27	710	4450	2550	5800	5.3	wet
	2	350	120	32	1420	4150	2650	5500	5.4	well drained
	3	250	190	38	2580	4150	3100	4200	4.5	wet
	4	1090	140	31	930	3900	2400	4300	5.2	wet
	5	770	160	29	1320	3400	2400	4600	4.2	well drained
	6	470	110	40	2020	4500	2650	4500	5.8	wet
	7	1200	180	28	2440	6250	2350	4450	4.9	wet
	8	970	170	38	710	3250	2100	4900	5.7	wet
	9	860	90	29	2900	4850	2950	4400	4.2	well drained
	10	650	120	31	1410	3800	2750	4600	3.3	well drained
	11	500	110	29	700	4050	2500	4700	5.9	wet
	12	500	210	31	2130	4850	2950	4200	4.5	wet
6	1	290	620	21	930	4125	1400	2350	2.0	47.83
	2	60	243	27	2420	3500	1950	2850	2.3	37.95
	3	210	326	25	320	5750	1800	3150	2.7	51.30
	4	70	187	29	1520	4375	2100	4000	2.8	35.59
	5	450	382	23	2150	4875	1800	2700	1.8	32.08
	6	90	76	18	2090	5750	2150	3050	2.4	27.09
7	1	1840	79	41	230	4500	1650	2550	1.1	51.01
	2	4450	168	49	340	4750	2250	3400	2.0	58.79
	3	720	84	37	---	5500	1350	2750	1.5	39.96
	4	5080	127	46	140	4875	2100	2900	1.9	63.09
	5	5040	118	47	---	3875	1850	2300	1.0	47.87
	6	5960	135	54	190	4750	2450	3050	1.8	63.89
8	1	4730	268	35	550	4875	1450	3250	2.5	49.83
	2	4080	513	30	500	4375	1300	2400	1.4	40.67
	3	3870	262	31	250	5000	1400	3150	2.0	43.98
	4	5670	559	28	1630	10375	1700	2450	2.2	71.10
	5	6170	248	35	2540	5750	1750	2000	1.4	41.28
	6	3730	522	32	760	4000	1400	2650	1.6	34.78

Table 25 (continued)

Pasture	Site	Al	Mn	Zn	Na	Ca	Mg	P	K	Soil Moisture
		ppm								%
9	1	1840	396	35	370	6000	1550	2950	1.5	39.25
	2	6330	871	35	920	3600	1900	2600	2.1	68.16
	3	2680	567	38	240	3800	1550	3250	2.2	37.26
	4	3240	354	27	840	4250	1450	2850	1.7	34.61
	5	5290	256	30	890	6300	1450	2450	1.3	42.73
	6	6780	242	34	1100	3800	1850	2900	2.0	35.50
10	1	4410	741	30	870	3875	1600	2850	2.3	44.90
	2	1620	665	26	320	3650	1200	3050	2.5	42.60
	3	2380	347	25	1140	5000	1400	2750	2.1	34.01
	4	3430	304	22	1080	1125	1700	2500	2.3	38.31
	5	3370	297	24	670	5625	1500	2500	2.2	46.87
11	1	1420	100	--	---	2550	2000	5500	3.5	40.43
	2	14500	140	--	---	3600	3350	4450	2.7	80.14
	3	620	90	--	---	2100	1650	5400	3.6	50.21
	4	2590	90	--	---	2450	1900	4300	2.6	---
	5	2280	80	--	---	1900	1650	3950	2.9	59.36
	6	490	80	--	---	1700	1600	4650	3.4	---
	7	980	80	--	---	2150	1650	4000	3.1	63.21
	8	530	90	--	---	2000	1600	4450	3.6	---
12	1	190	300	33	---	6100	1600	2000	0.9	---
	2	4400	180	49	---	5900	2025	2600	1.4	---
	3	190	200	31	---	4450	1530	1950	1.1	---
	4	180	340	45	---	6200	1925	2800	1.3	---
	5	960	170	42	---	6300	1545	2600	1.5	---
	6	280	200	37	---	3450	1420	2300	1.4	---
	7	890	200	42	---	4700	1590	1950	0.9	---
	8	200	240	33	---	6100	1600	1950	0.8	---
	9	240	320	34	---	6150	1665	2300	0.9	---
	10	600	300	37	---	6650	1735	1850	0.7	---

Table 26. The chemical analysis of soil samples collected in pastures grazed by animals with a diagnosis of grass tetany.

Pasture	Site	Exchangeable Al	Mn	Na	Ca	Mg	P	K	pH
		meq/100g	ppm						
1	2+	--++	78	57	1220	153	31	88	7.0
2	1	--	134	69	500	120	177	181	5.7
	2	--	77	68	630	175	229	134	6.0
	3	--	308	67	110	26	115	118	5.1
3	1	--	96	72	660	159	24	111	5.6
	2	--	37	74	1095	203	24	173	6.1
	3	--	55	67	870	179	29	90	6.0
4	1	--	25	58	1420	299	215	225	5.4
	2	--	3;	66	1310	285	201	160	5.5
	3	--	28	72	1185	271	186	174	5.2
	4	--	23	61	1445	279	234	258	5.5
5	1	--	19	47	1450	231	86	115	6.7
	2	--	13	45	980	180	86	119	6.5
	3	--	32	42	620	123	115	84	5.7
	4	--	28	45	1145	171	86	159	6.3
	5	--	44	40	650	108	115	181	5.9
	6	--	26	46	1120	136	105	132	6.2
	7	--	53	43	1325	119	100	78	6.8
	8	--	49	46	1160	181	91	212	6.5
	9	--	20	49	1275	193	57	157	6.8
	10	--	15	43	910	151	76	142	6.3
	11	--	41	38	1040	142	62	137	6.5
	12	--	73	44	810	126	62	143	6.1
6	1	0.0	152	79	670	74	48	76	5.9
	2	0.0	71	61	940	96	55	72	5.9
	3	0.0	53	65	920	73	67	112	6.2
	4	0.2	29	63	810	101	81	58	5.8
	5	0.0	99	61	630	38	41	41	6.2
	6	0.0	17	70	1750	96	57	48	6.8
7	1	0.0	22	65	4000+	870	249	412	6.0
	2	0.0	21	74	4000+	837	201	487	5.8
	3	0.0	18	64	4000+	869	163	435	5.9
	4	0.0	31	65	4000+	783	258	500+	6.2
	5	0.0	26	51	4000+	873	165	448	6.0
	6	0.0	12	61	4000+	940	330+	382	5.7
8	1	0.0	60	88	1335	194	201	135	6.2
	2	0.0	145	80	1245	114	76	109	6.8
	3	0.0	63	55	2145	107	96	172	7.2
	4	0.0	110	84	1679	115	41	115	6.7
	5	0.0	47	62	2170	175	48	120	7.3
	6	0.0	142	59	1195	128	55	79	6.5

Table 26 (continued).

Pasture	Site	Exchangeable Al	Mn	Na	Ca	Mg	P	K	pH
		meq/100g	ppm						
9	1	0.0	132	57	1090	99	81	96	6.4
	2	0.0	102	49	880	196	35	110	5.6
	3	0.1	92	48	710	75	143	211	5.8
	4	0.0	136	70	1310	87	67	145	7.0
	5	0.0	38	49	1335	125	31	81	6.9
	6	0.0	52	61	2040	277	57	280	6.6
10	1	0.0	101	72	990	233	33	94	5.6
	2	0.0	164	65	850	118	57	131	6.6
	3	0.0	144	57	2185	106	43	123	7.6
	4	0.0	69	77	1465	154	29	105	6.7
	5	0.0	69	100	1840	126	48	98	7.1
11	1	0.0	33	61	2960	653	133	500+	6.6
	2	0.0	55	108	3950	755	300	500+	6.8
	3	0.0	40	56	3335	672	152	500+	6.5
	5	0.0	56	71	3340	690	90	371	6.1
	7	0.0	38	83	3385	689	109	305	6.4
12+++									

+ Site numbers correspond to forage sample site numbers in Table 25.

++Indicates missing data.

+++Animals at this location were on a hay diet.

Table 27. Analysis of serum samples from lactating cows using two dilution techniques.

Day	Animal No.	distilled water		distilled water with 5% HCl	
		Mg	Ca	Mg	Ca
		ppm			
1	343	13.20	88.00	--	--
	109	17.27	81.40	--	--
	134	19.36	91.30	--	--
	342	15.40	71.50	--	--
	236	13.09	88.00	--	--
	328	17.60	93.50	--	--
	688	20.68	86.90	--	--
	694	15.95	81.40	--	--
2	343	8.14	83.60	--	--
	109	15.18	83.60	--	--
	134	17.05	90.20	--	--
	342	12.76	78.10	--	--
	236	9.02	78.10	--	--
	328	17.49	88.00	--	--
	688	17.82	91.30	--	--
	694	14.63	85.80	--	--
3	343	8.03	79.20	--	--
	109	14.63	84.70	--	--
	134	13.64	86.90	--	--
	342	13.42	73.70	--	--
	236	8.69	74.80	--	--
	328	17.93	84.70	--	--
	688	19.36	90.20	--	--
	694	17.60	83.60	--	--
4	343	10.01	81.40	10.56	86.90
	109	15.51	83.60	16.72	86.90
	134	12.87	84.70	13.09	86.90
	342	14.85	70.40	15.84	82.50
	236	10.34	68.20	11.55	80.30
	328	19.36	75.90	20.24	84.70
	688	21.89	90.20	22.88	96.80
	694	19.69	81.40	21.34	90.20
5	343	8.25	--	9.02	73.70
	109	13.75	--	15.07	84.70
	134	10.78	--	12.10	84.70
	342	14.19	--	16.50	75.90
	236	10.67	--	11.44	79.20
	328	18.37	--	--	--
	688	20.90	--	21.23	59.40
	694	18.92	--	20.90	89.10

Table 27 (continued)

Day	Animal No.	distilled water		distilled water with 5% HCl	
		Mg	Ca	Mg	Ca
		ppm			
6	343	9.79	--	11.00	89.10
	109	14.30	--	15.07	81.40
	134	11.32	--	--	--
	342	16.61	--	18.48	86.90
	236	11.66	--	12.21	81.40
	328	17.82	--	20.02	80.30
	688	22.99	--	24.75	102.30
	694	19.58	--	22.00	96.80
7	343	--	--	13.42	91.30
	109	--	--	16.39	86.90
	134	--	--	17.38	95.70
	342	--	--	18.48	86.90
	236	--	--	13.97	96.80
	328	--	--	20.68	83.60
	688	--	--	23.32	97.90
	694	--	--	23.21	91.30
8	343	--	--	10.12	86.90
	109	--	--	12.65	92.40
	134	--	--	10.23	89.10
	342	--	--	16.94	83.60
	236	--	--	11.99	85.80
	328	--	--	16.83	94.60
	688	--	--	24.31	95.70
	694	--	--	22.00	91.30

LITERATURE CITED

1. Allcroft, W.M. and H.H. Green. 1938. Seasonal hypomagnesemia of the bovine without clinical symptoms. J. Comp. Pathol. Therap. 51:176-191.

2. Baker, R.M., R.C. Boston, T.E. Boyes, and D.D. Leaver. 1976. The experimental induction of hypomagnesemia in the adult wether sheep. Proc. Aust. Soc. Anim. Prod. 11:381-384.

3. Beeson, K.C. 1959. In Magnesium and Agriculture (G.C. Anderson, E.M. Jencks, and D.J. Horvath, eds.) pp. 1-11. West Virginia University, Morgantown, West Virginia.

4. Bertoni, G., M.J. Watson, G.P. Savage, D.G. Armstrong. 1976. The movement of minerals in the digestive tract of dry and lactating Jersey cows. 1. Net movements of Ca, P, Mg, Na, K, and Cl. Zootecnica e Nutrizione Animale 2(2) 107-118.

5. Black, C.A. (Ed.). 1965. Methods of Soil Analysis; part 1, Physical and Mineralogical Properties, including Statistics of Measurement and Sampling. ASA, Madison, Wisc.

6. Blakemore, F., J.A. Nicholson and J. Stewart. 1937. Some effects of a high manganese content in the diet of animals, with special reference to lactation tetany. Vet. Rec. 49:415-422.

7. Bowen, G.D. 1973. Mineral nutrition of ectomycorrhizae. pp. 151-205. In G.C. Marks and T.T. Kozlowski (ed.) Physiology and ecology of ectomycorrhizae. Academic Press. New York, N.Y.

8. Butler, E.J. 1963. The mineral element content of spring pasture in relation to the occurrence of grass tetany and hypomagnesaemia in dairy cows. J. Agric. Sci. 60:329-340.

9. Clarkson, D.T. 1966. Effect of aluminum on some trivalent metal cations on cell division in the root apices of Allium cepa. Ann. Bot. (London) 29:309-315.

10. Clarkson, D.T. 1969. Metabolic aspects of aluminum toxicity and some possible mechanisms for resistance. pp. 381-397. In I.H. Rorison (ed.) Ecological aspects of mineral nutrition of plants. Blackwell Scientific Publications, Oxford and Edinburgh.

11. Coles, E.H. 1974. Veterinary clinical pathology. W.B. Saunders Co. Phil. Pa.

12. Cooper, H.P., W.R. Paden, W.H. Garman. 1947. Some factors influencing the availability of magnesium in soil and the magnesium content of certain crop plants. Soil Sci. 63:27-42.

13. Crookshank, H.R. and F.H. Sims. 1955. Serum values in wheat pasture poisoning cases. J. Anim. Sci. 14:964-969.

14. Cunningham, G.N., M.B. Wise, and E.R. Barrick. 1966. Effect of high dietary levels of manganese on the performance and blood constituents of calves. J. Anim. Sci. 25:532-538.

15. David, L. Chapuis, M.C. Collombil, C. Racle, B. Racle, P. Francois. 1975. The pathogenic mechanism of the hypocalcaemia in primary hypomagnesaemia. Demonstration of a block in secretion of parathyroid hormone. Archives Francaises de Pediatrie. 1975. 32:803-813.

16. Davis, R.E. 1959. In Magnesium and agriculture. (G.C. Anderson, E.M. Jencks, and D.J. Horvath, eds.), pp. 154-158. West Virginia University, Morgantown, West Virginia.

17. DeKock, P.C. and R.L. Mitchell. 1957. Uptake of chelated metals by plants. Soil Sci. 84:55-62.

18. Dennis, Everett J. 1971. Magnesium deficiency and grass tetany: is aluminum a key? Fert. Sol. Mar/Apr. 1971. pp. 44-54.

19. Dishington, I.W. 1965. Changes in serum magnesium levels of ruminants, as influenced by abrupt changes in the composition of the diet. Acta. Vet. Scand. 6:150-77.

20. Elkins, C.B. and C.S. Hoveland. 1977. Soil oxygen and temperature effect on tetany potential of three annual forage species. Agron. J. 69:626-8.

21. Elkins, Charles B., R.L. Haaland, C.S. Hoveland, and W.A. Griffey. 1978. Grass tetany potential of tall fescue as affected by soil O_2. Agron. J. 70:309-311.

22. Epstein, F.H. (ed.) 1976. Parathyroids, vitamin D, calcitonin, calcium and magnesium. In Yearbook of Medicine. D.E. Rogers, R.M. DesPrez, P. Heller, E. Brownwald, N.J. Greenberger, P. Bondy and F.H. Epstein (eds.). Yearbook Medical Publishers, Inc. Chicago.

23. Fain, P., J. Dennis, and F.G. Harbaugh. 1952. The effect of added manganese in feed on various mineral components of cattle blood. Am. J. Vet. Res. 13:348.

24. Field, A.C. and C.S. Munro. 1977. The effect of site and quantity on the extent of absorption of Mg infused into the gastro-intestinal track of sheep. J. Agric. Sci., Camb. 89:365-371.

25. Flink, E.B. 1976. Magnesium deficiency and magnesium toxicity in man. In Trace elements in human health and disease. Vol. II. Essential and toxic elements. pp. 1-21.

26. Fontenot, J.P. 1972. Magnesium in ruminant animals and grass tetany. In J. Benton Jones, Jr., M.C. Blout, and S.R. Wilkinson (eds.) Magnesium in the environment: soils, crops, animals, and man. Taylor Publishing Co., Reynolds, Ga.

27. Forbes, A.J. 1972. Clinical cases of seasonal grass tetany in lactating beef cows. Aust. Vet. J. 48:444-8.

28. Foy, C.D., and A.L. Fleming. 1978. The physiology of plant tolerance to excess available aluminum and manganese in acid soils. In Crop tolerance to suboptimal land conditions. ASA Special Publication No. 32. pp. 301-328.

29. Gallup, W.D., A.B. Nelson and A.E. Darlow. 1952. Forage manganese as a possible factor affecting calcium and phosphorus metabolism of range beef cattle. J. Anim. Sci. 11:783.

30. Gerdemann, J.W. 1968. Vescicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-418.

31. Gillingham, J.T., and N.R. Page. 1965. Influence of anions on the uptake of calcium and magnesium by plants and on calcium movement in soils. Agron. J. 57:83-88.

32. Grace, N.D. and J.C. MacRae. 1972. Influence of feeding regimen and protein supplementation on the sites of net absorption of magnesium in sheep. Br. J. Nutr. 27:51-55.

33. Grunes, D.L., P.R. Stout, and J.R. Brownell. 1970. Grass tetany of ruminants. Adv. Agron. 22:331-374.

34. Harley, J.L. 1969. The biology of mycorrhiza. 2nd Ed. Leonard Hill Ltd., London, England.

35. Head, M.J., J.A.F. Rook. 1957. Some effects of spring grass on rumen digestion and the metabolism of the dairy cow. Proc. Nutr. Soc. 16:25-30.

36. Hemingway, R.G. and N.S. Ritchie. 1965. The importance of hypocalcaemia in the development of hypomagnesaemic tetany. Proc. Nutr. Soc. 24:54-62.

37. Hemingway, R.G., N.S. Ritchie, Nora A. Brown, J.N. Peart. 1965. Effects of grazing management on plasma calcium and magnesium concentrations of ewes in early lactation. J. Agric. Sci., Camb. 64:109-13.

38. Hemingway, R.G., N.S. Ritchie, A.R. Rutherford, and G.M. Jolly. 1963. Effects of potassium fertilizers, age of ewe, and small magnesium supplementation on blood magnesium and calcium levels of lactating ewes. J. Agric. Sci. 60:307-312.

39. Hjerpe, C.A. 1964. Grass tetany in California cattle. J. Am. Vet. Med. Ass. 144:1406.

40. Hobbs, C.S., R.P. Moorman, J.M. Griffith, J.L. West, G.M. Merriman and C.C. Chamberlain. 1954. Fluorosis in cattle and sheep. Tenn. Agr. Exp. Sta. Bull. 235.

41. Hoflund, S. and H. Hedstrom. 1949. The connection between deficiency diseases and disturbances in rumen digestion. Acta Agric. Suec. 3:121-34.

42. Holevas, C.D. 1966. The effect of a vesicular-arbuscular mycorrhiza on the uptake of soil phosphorus by strawberry (Fragaria sp. var. Cambridge Favourite). J. Hort. Sci. 41:57-64.

43. Hutchinson, G. Evelyn. 1945. Aluminum in soils, plants, and animals. Soil Sci. 60:29-40.

44. Jones, J.H. 1938. The metabolism of calcium and phosphorus as influenced by the addition to the diet of salts of metals which form insoluble phosphates. Amer. J. Physiol. 124:230.

45. Jones, L.H. 1961. Aluminum uptake and toxicity in plants. Plant and Soil 14:297-310.

46. Karlen, D.L., R. Ellis, Jr., D.A. Whitney and D.L. Grunes. 1978. Influence of soil moisture and plant cultivar on cation uptake by wheat with respect to grass tetany. Agron. J. 70:918-21.

47. Kemp, A. 1960. Hypomagnesaemia in milking cows: The response of serum magnesium to alterations in herbage composition resulting from potash and nitrogen dressing on pasture. Neth. J. Agric. Sci. 8:281-304.

48. Kemp, A., and M.L. 't Hart. 1957. Grass tetany in grazing milking cows. Neth. J. Agric. Sci. 5:4-17.

49. Kemp, A., W.B. Deijs, O.J. Hemkes, A.J.H. Van Es. 1961. Hypomagnesaemia in milking cows: intake and utilization of magnesium from herbage by lactating cows. Neth. J. Agric. Sci. 9:134-149.

50. Kemp, A., A. Th. van't Klooster, P.A.M. Rogers, and J.H. Geurink. 1973. Studies on the amount and composition of digesta flowing through the duodenum of dairy cows. 2. Site of net absorption of magnesium and calcium from the alimentary tract. Neth. J. Agric. Sci. 21:44-55.

51. Leggett, J.E., D.B. Egli, and L. Bush. 1977. Effect of root temperatures on growth and cation composition of Festuca arundinaceae Schreb. Agron. J. 69:723-4.

52. Lentz, D.E., F.C. Madsen, J.K. Miller, S.L. Hansard. 1976. Effect of potassium and hypomagnesaemia on insulin in the bovine. J. Anim. Sci. 43:1082-1087.

53. l'Estrange, J.L. and R.F.E. Axford. 1964. A study of magnesium and calcium metabolism in lactating ewes fed a semi-purified diet low in magnesium. J. Agric. Sci., Camb. 62:353-68.

54. l'Estrange, J.L., and R.F.E. Axford. 1964. A study of serum mineral changes in lactating Welsh mountain ewes under different grazing conditions with special reference to hypomagnesaemia. J. Agric. Sci., Camb. 62:341-51.

55. Maas, E.V., D.P. Moore, and B.J. Mason. 1968. Manganese absorption by excised barley roots. Plant Physiol. 43:527-30.

56. Maas, E.V., D.P. Moore, and B.J. Mason. 1969. Influence of calcium and magnesium on manganese absorption. Plant Physiol. 44:796-800.

57. Madsen, F.C., D.E. Lentz, J.K. Miller, D. Lowrey-Harden, S.L. Hansard. 1976. Dietary carbohydrate effects upon magnesium metabolism in sheep. J. Anim. Sci. 42:1316-1322.

58. Magistad, O.C. 1925. The aluminum content of the soil solution and its relation to soil reaction and plant growth. Soil Sci. 20:181.

59. Maiden, J.H. and H.G. Smith. 1895. On a natural deposit of aluminum succinate in the timber of Grevillea robusta R. Br. Proc. Roy. Soc. N.S.W. 29:325-35.

60. Marshak, R.R. 1959. In "Magnesium and agriculture." Symp. W. Va. Univ. pp. 169-78.

61. Martens, H., J. Harmeyer, G. Breves, and H. Scholz. 1976. Magnesium absorption "in vitro" through rumen mucosa of sheep. Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde 37(1) 44-52.

62. Mayland, H.F., D.L. Grunes, V.A. Lazer. 1976. Grass tetany hazard of cereal forage based upon chemical composition. Agron. J. 68:665-667.

63. McConaghy, S., J.S.V. McAllister, J.R.Todd and J.E.F. Rankin. 1963. The effects of magnesium compounds and of fertilizers on the mineral composition of herbage and on the incidence of hypomagnesaemia in dairy cows. J. Agric. Sci. 60:313-328.

64. McIntosh, S., P. Crooks, and K. Simpson. 1973. The effects of applied N K and Mg on the distribution of Magnesium in the plant. Plant and Soil 39:389-97.

65. McNaught, K.J., F.D. Dorofaeff, and J. Karlousky. 1969. Effect of magnesium fertilizers and season on level of inorganic nutrients in a pasture on Hamilton clay loam. N. Z. J. Agric. Res. 11:533-50.

66. Mederski, H.G. and D.J. Hoff. 1958. Manganese deficiency in soybeans. pp. 99-108. In C. A. Lamb et al. (ed.) Trace elements. Academic Press, Inc., New York.

67. Mendalle, R., C. Waterhouse, T.J. Hahn. 1976. Vitamin D resistance in magnesium deficiency. Amer. J. of Clinical Nut. 29:854-858.

68. Mershon, M.M. 1959. Tetany in cattle on winter rations. Part II. Stresses and mineral metabolism. J. Am. Vet. Med. Ass. 135:435-9.

69. Metson, A.J., W.H.H. Saunders, T.W. Collie, V.W. Graham. 1966. Chemical composition of pastures in relation to grass tetany in beef breeding cows. N. Z. J. of Agric. Res. 9:410-36.

70. Meyer, H. 1960. Magnesiumstoffwechsel, magnesiumbedarf, und magnesiumversorgung bei den haustieren. M. & H. Schaper Co., Hannover, Germany.

71. Meyer, H. 1976. The physiology of magnesium metabolism in ruminants. In Magnesium in Ruminant Nutrition. Israel Chemicals Ltd., Tel-Aviv, Israel.

72. Miller, J.K., D.E. Lentz, F.C. Madsen, S.L. Hansard. 1976. Relationship of dietary carbohydrate, magnesium, and potassium to grass tetany. Tenn. Farm and Home Science. April/June 2-4.

73. Moore, D.P., R. Overstreet, and L. Jacobson. 1961. Uptake of magnesium and its interaction with calcium in excised barley roots. Plant Physiol. 36:290-95.

74. Mosse, B. 1972. Effects of different Endogone strains on the growth of Paspalum notatum. Nature 239:221-23.

75. Mosse, B. 1972. The influence of soil type and Endogone strain on the growth of mycorrhizal plants in phosphate-deficient soils. Rev. Ecol. Biol. Soil 9:592-37.

76. Mosse, B. 1973. Advances in the study of vesicular arbuscular mycorrhiza. Annu. Rev. Phytopath. 11:171-96.

77. National Research Council. 1971. Nutrient requirements of dairy cattle. No. 3. National Academy of Sciences, Washington, D.C.

78. National Research Council. 1976. Nutrient requirements of beef cattle. No. 4. National Academy of Sciences, Washington, D.C.

79. The nutrient requirements of farm livestock. No. 2. Ruminants, technical reviews and summaries. 1965. Agricultural Research Council. London, Eng. p. 60.

80. Ondieicka, R., J. Kortus, and E. Ginter. 1971. In "Intestinal absorption of metal ions, trace elements and radionuclides." (S.C. Skoryna and D. Waldron-Edward, eds.) p. 293. Pergamon, Oxford.

81. Pauli, J.V. and T.F. Allsop. 1974. Plasma and cerebrospinal fluid magnesium, calcium and potassium concentrations in dairy cows with hypomagnesaemic tetany. N. Z. Vet. J. 22:227-231.

82. Pfeffer, E., A. Thompson, and D.G. Armstrong. 1970. Studies on intestinal digestion in the sheep. 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. Br. J. Nutr. 24:197-204.

83. Rhue, R.D. 1976. The time concentration of Al toxicity in wheat root meristems. Ph.D. Thesis, Dep. of Soil Sci. Oregon State Univ., Corvallis, Oreg.

84. Robinson, N.W., Sam L. Hansard, D.M. Johns and G.L. Robertson. 1960. Excess dietary manganese and feed lot performance of beef cattle. J. Animal Sci. 19:1290 (Abstr.)

85. Rogers, P.A.M., and A. Th. van't Klooster. 1969. The fate of Na, K, Ca, Mg and P in the digesta. Meded. Landbhoogesch. Wageningen. 69:(11) 26-39.

86. Rook, J.A.F. and C.C. Balch. 1958. Magnesium metabolism in the dairy cow. J. Agr. Sci. (Camb.) 51:199-207.

87. Rorison, I.H. 1965. The effect of aluminum on the uptake and incorporation of phosphate by excised sanfoin roots. New Phytol. 64:23-27.

88. Ross, J.P. and J.A. Harper. 1970. Effect of endogone mycorrhiza on soybean yields. Phytopath. 60:1552-1556.

89. Rubins, E.J., and Hagstrom, G.R. 1959. Determination of aluminum and iron in plant tissue. J. Agric. Food Chem. 7:722-724.

90. Russell, E. Walter. 1961. Soil conditions and plant growth. 9th ed. John Wiley and Sons Ltd., New York.

91. Salmon, R.C. 1963. Magnesium relations in soils and plants. Jl. Sci. Food Agr. 14:605-610.

92. Shils, Maurice E. 1976. Magnesium deficiency and calcium and parathyroid hormone interrelations. In Trace elements in human health and disease. Vol. II. Academic Press, New York, pp. 23-46.

93. Shorland, F.B. 1934. The estimation of Al in pastures with special reference to soil contamination. Proc. Roy. Soc. N. Z. 64:35-50.

94. Sjollema, B. 1928. Over het Wezen en de Therapie van Paresis puerperalis. T. Diergeneesk. 55:1017-1036, 1085-1105, 1121-1122, 1187-1205.

95. Sjollema, B. 1930. On the nature and therapy of grass staggers. Vet. Rec. 10:425-431 and 450-454.

96. Small, J. 1946. pH and plants. Bailliere, Tindall and Cox, London.

97. Smith, H.G. 1903. Aluminum, the chief inorganic element in a proteaceous tree, and the occurrence of aluminum succinate in trees of this species. Proc. Roy. Soc. N. S. W. 37:107-20.

98. Sorenson, John R.J. 1977. Aluminum in relation to the environment and human health. In Environmental Biogeochemistry. Vol. 2. Metals transfer and ecological mass balances. Chapter 27, p. 427-450 (Ed. Jerome O. Nriagu) Ann Arbor Sci., Ann Arbor, Mich.

99. Storry, J.E. and J.A.F. Rook, 1963. Magnesium metabolism in the dairy cow. V. Experimental observations with a purified diet low in magnesium. J. Agric. Sci. 61:167-171.

100. Stuart, D.M., H.F. Mayland, and D.L. Grunes. 1973. Seasonal changes in trans-aconitate and mineral composition of Crested Wheatgrass in relation to grass tetany. J. Range Man. 26:113-116.

101. Swan, J.B. and N.D. Jamieson. 1956. Studies on metabolic disorders in dairy cows. I. Diagnostic methods and a survey of clinical cases. N. Z. J. Sci. Technol. A38:137-51.

102. Swan, J.B., and N.D. Jamieson. 1956. Studies on metabolic disorders in dairy cows. III. The effects on after-calving underfeeding and of thyroprotein dosing on the level of serum magnesium in dairy cows. N.Z. J. Sci. Technol. A38:363-82.

103. 't Hart, M.L. 1960. In Conference on hypomagnesaemia (Anonymous, ed.), pp. 88-95. Victoria Hall, Southhampton Row, London.

104. Thompson, A., S.L. Hansard and M.C. Bell. 1959. The influence of aluminum and zinc upon the absorption and retention of calcium and phosphorus in lambs. J. Anim. Sci. 18:187.

105. Tinker, P.B.H. 1975. Effects of vesicular-arbuscular mycorrhizas on higher plants. pp. 325-349. In D.G. Jennings and D.L. Lee (eds.) Symbiosis. 29th Symp. Soc. Exp. Biol. Cambridge Univ. Press, London, England.

106. Tomas, F.M. and B.J. Potter. 1976. The site of magnesium absorption from the ruminant stomach. Br. J. Nutr. 36:37-45.

107. Truog, Emil, G.C. Gerloft, R.J. Goates and K.C. Berger. 1947. Magnesium-phosphorus relationships in plant nutrition. Soil Sci. 63:19-25.

108. Underwood, E.J. 1956. Trace Elements in human and animal nutrition. Academic Press Inc., New York.

109. Underwood, E.J. 1966. The mineral nutrition of livestock. pp. 63-79. Food Agr. Organ. United Nations and the Commonwealth Agr. Bur. Gt. Brit.

110. Underwood, E.J. 1977. Trace elements in human and animal nutrition. 4th ed. Academic Press, New York.

111. Valdivia, R., C.B. Ammerman, C.J. Wilcox and P.R. Henry. 1978. Effect of dietary aluminum on animal performance and tissue mineral levels in growing steers. J. Anim. Sci. 47:1351-6.

112. Voisin, A. 1963. "Grass Tetany." Thomas, Springfield, Ill.

113. Webb, L.J. 1954. Aluminum accumulation in the Australian-New Guinea flora. Aust. J. of Bot. 2:176.

114. White, J.B. 1961. Clinical Hypomagnesaemia. Proceedings British Vet. Ass. Conf. Hypomagnesaemia. pp. 39-44.

115. Wilkinson, S.R. and J.A. Stuedeman. 1979. Grass tetany. Ch. 5. ASA Special Publication No. 35. Madison, WI 53711.

VITA

Vivien Gore Allen was born on March 31, 1940 in Nashville, Tennessee. She is married to the Reverend Harry E. Allen, Jr. and has three sons, Evans, Lawton and Stephen.

She was graduated from Peabody Demonstration School, Nashville, in 1958.

She entered the University of Tennessee, at Martin, in 1958 and was graduated in 1962 receiving the Bachlor of Science degree in General Agriculture.

In 1972 she entered Louisiana State University as a graduate student in the Department of Agronomy. She was graduated in 1973 receiving a Master of Science degree with a major in Agronomy and a minor in Animal Science.

Since 1973 she has been manager-owner of Feliciana Farms in Franklin County, Tennessee.

She reentered Louisiana State University in 1975 and is presently a candidate for the degree of Doctor of Philosophy in the Department of Agronomy.

EXAMINATION AND THESIS REPORT

Candidate: Vivien Gore Allen

Major Field: Agronomy

Title of Thesis: Aluminum and Manganese in the Etiology of Grass Tetany.

Approved:

Donald L. Robinson
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Joe E. Sedberry, Jr.

Edward P Dunigan

F. Glen Hembry

C. L. Mondart Jr.

V. M. Selim

Date of Examination:

November 27, 1979